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CRYSTALS AND PHOTONS*

By Sir C. V. Raman

My first duty on this occasion is to express our gratitude to the authorities of the Andhra University for their generous sympathy and support to the work of the Academy and the opportunity they have afforded us of having our Annual Meeting in a truly academic atmosphere set amidst the beautiful scenery of Waltair. We appreciate very much the warmth of the welcome we have received. Our gathering here gives the Fellows of the Academy an opportunity of visiting the Andhra University at a time when many new developments are receiving attention, and of meeting men like Professor Bhagavantam, Professor Seshadri and Dr. Nagendra Nath, whose research papers have filled the pages of the *Proceedings* of the Academy and whose work has shed lustre on the University. They have, like our many other Fellows in other parts of India, given ungrudgingly of their time and energy for the welfare of the Academy. Our special thanks are due to them and other local Fellows and to the Chairman and members of the Reception Committee for having worked to make this meeting a success.

In my Presidential Address last year at Bangalore, a long-term programme of research on the physics of crystals was outlined and put forward as likely to yield valuable results for our knowledge of the solid state. Looking over the *Proceedings* of the Academy for the last twelve months, I find that fourteen papers on crystal physics (listed at the end of this address) have appeared in it, of which three are from Waltair and the rest from Bangalore. We have no reason to be dissatisfied with the progress made so far, and indeed it may be claimed that some of these papers deal with the problems of the solid state from a quite novel standpoint and open up new pathways of investigation. I propose in this address to survey broadly the field of research dealt with in these papers which may be designated as the newer crystal optics based on the ideas of the quantum theory, to distinguish it from the older optics which considers the effects of passage of radiation through crystals on the wave-principles. The quantum optics links together the phenomena observed with infra-red radiation, visible light and X-rays in a remarkable way, and reveals the existence of a new type of X-ray reflection in crystals.

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* Presidential Address delivered at the Annual Meeting of the Indian Academy of Sciences held on the 27th December 1940, at the Andhra University, Waltair.

As is well known, the behaviour of a crystal with respect to common light is intimately related with the geometric symmetry of the system to which it belongs. Rock-salt and diamond, for example, which are cubic crystals are isotropic or singly refracting; calcite and sodium nitrate which are rhombohedral crystals are doubly refracting but optically uniaxial; aragonite and barite which are orthorhombic are doubly refracting but optically biaxial. These characters of the respective crystals are determined by the optical polarisability of the substance in different directions. The polarisability may be geometrically represented by a surface which is a sphere for an isotropic crystal, a spheroid of revolution for a uniaxial crystal, and an ellipsoid with three unequal axes for a biaxial crystal. The polarisability is a bulk or molar property, which is intimately connected with the atomic architecture of the crystal, that is to say, by the properties of the atoms or ions or molecules composing the crystal, and by the manner in which they are spaced, orientated and linked together. The modern techniques for growing large crystals enable us to study and exhibit the very striking optical behaviour of many common substances. Sodium nitrate, for instance, can be grown into large crystals, and a block of it shows a birefringence which is even more obvious and striking than that of calcite. Still better is naphthalene which is a biaxial crystal and shows an extremely large birefringence. *The angles of internal and external conical refraction of naphthalene are about* 14°, *and are thus eight times larger than those of aragonite with which conical refraction is usually exhibited.* Needless to say, conical refraction as observed with a piece of naphthalene is far more striking than that exhibited by aragonite. As was shown by Bhagavantam in the very first paper he published as a research student, the strong birefringence of naphthalene is closely connected with the very great optical anisotropy of the molecules as well as the special orientation of the molecules in the crystal.

The classical optics of crystals prefers to ignore the atomic architecture of the solid and regards the substance as a continuum with specific molar properties. The crystal functions as a pathway for the passage of the radiation, taking part in its propagation but remaining essentially undisturbed in the process. Even when, as frequently happens, the radiation is partially absorbed by the crystal, this is fitted mathematically into the wave-picture by making the optical constants of the substance complex quantities instead of real numbers. Such a wave-picture is a satisfactory description of the phenomena which it is intended to cover. But it is not a complete description of the facts. This was shown clearly by the studies on the scattering of light in crystals commenced by the writer in the year 1921 and culminating in the discovery of the effect of a change of frequency described in the lecture on " A New Radiation " in February 1928. The method of

observation described in that lecture involves the use of monochromatic light and a spectroscope. To take a specific example, we allow the beam of light from a mercury arc lamp to pass through a crystal of diamond. Focussing an image of the illuminated diamond on the slit of a spectroscope, we observe in the light scattered in the substance of the diamond, new lines not present in the incident radiation. Corresponding to each monochromatic line in the incident radiation, there is a second and additional line, the wave-number of which is less by 1332 per centimetre than for the incident light. A third and much feebler line of which the wave-number *exceeds* that of the incident light by 1332 per centimetre is also weakly recorded in the spectrum. Numerous such studies of the scattering of light in crystals have been made since they were first described. It is abundantly clear from the observations that they cannot be explained on the basis of the ideas of the classical wave-theory alone. It is necessary to introduce the concept of the photon or a quantum of radiation, the energy of which is proportional to the frequency of the corresponding waves. The change of frequency as actually observed appears on the quantum theory as due to an exchange of energy between the photon and the crystal, a diminution of frequency if the photon is the donor and the crystal the acceptor, and an increase if the reverse is the case.

The investigation of light scattering in crystals is a powerful method for the study of the solid state. But it is beset by peculiar experimental difficulties. Theory indicates that a perfectly homogeneous crystal would scatter no light at all; the diffusion of light theoretically possible is that due to the vibrations of the crystal lattice excited by the incident light and occurs in every case with a change of frequency. Unfortunately, however, actual crystals are far from being perfect. Internal flaws and surface imperfections result in a strong scattering of light with unaltered frequency. Theoretically this should not trouble us at all, but practically it does trouble us very much. The difficulty arises from the fact that the incident light is not truly monochromatic and usually contains additional components and some continuous spectrum. If the crystal is not clear, or if the spectrograph gives appreciable coma or scattered light of its own, there is not much hope of getting useful results. Truly monochromatic light sources, clear crystals of sufficient size with polished faces, and spectrographs of high optical perfection and illuminating power are needed for such work. When these requirements are satisfied, results of great interest and value are forthcoming.

To appreciate the significance of the results obtained in such studies, we must remember that a crystal is not a mere geometric array of atoms, molecules or ions in space, but is a coherent structure of such particles having identical properties which are held together by powerful forces, thereby

forming a rigid solid. It is inappropriate to consider the observed results in terms of the vibrations of the individual ions, atoms or molecules in a crystal. For, it is obviously not possible for any one particle in a crystal to vibrate without setting all the other particles in resonant vibration. To understand the optical behaviour of a crystal rightly, we must consider it as a whole and set ourselves to discover the various modes of vibration of which its structure is capable. These vibrations divide themselves into two classes. In the first class of vibration, we can ignore the atomic architecture of the crystal and regard it as an elastic solid traversed by acoustic waves. The frequency of such vibration may be anything from zero upwards to a value so high that the corresponding wave-length becomes comparable with the spacing of the atomic planes in the crystal. In the second class of vibration, we are dealing with movements which can only be understood or described in terms of the atomic architecture of the crystal. Such vibrations are referred to as the optical vibrations of the lattice. Some of these optical vibrations may have no counterpart at all for the substance in a fluid state and arise as a consequence of the crystal fields which bind the chemical units together into a rigid and ordered assemblage. Some of the optical vibrations, however, may have frequencies roughly corresponding to those characteristic of the ions or molecules in the fluid state. Even so, they cannot be identified with the vibrations of the individual chemical units. The optical vibrations—quite as much as the acoustic ones—are characteristic of the crystal structure and not of the individual particles present in the lattice cells.

The simplest way in which we may picture an optical vibration of a crystal lattice is to imagine the interpenetrating lattices of the simplest type present in the crystal, each carrying a single atom at the lattice points, to oscillate as rigid units relatively to each other, the centre of the inertia of the whole assembly remaining at rest. In such an oscillation, the crystal would throughout remain a homogeneous structure, but the relative positions of the atoms in its lattice cells would vary periodically with time. Such physical properties as the electric dipole moment, optical polarisabilities, and structure-amplitudes which determine the behaviour of radiation in various ranges of frequency in its passage through the crystal—would, therefore, also vary periodically with the frequency of the oscillation. It can be readily seen, however, that optical vibrations of the general description indicated would also be possible in which the phase of the oscillation changes slowly from place to place within the crystal. If this phase varies regularly in such manner as to repeat itself over a series of regularly spaced planes, the spacing of such planes is the phase wave-length of the optical vibration. An infinite phase-wave-length indicates a vibration identically the same throughout the crystal, and the optical frequency has then its limiting value.

The importance of the considerations set out above becomes clear when we consider the effect of radiations falling on a crystal. We may first refer to the case of a beam of infra-red radiation incident normally on the surface of a crystal. It is a well-known experimental fact that if the frequency of such radiation is within certain ranges characteristic of the particular substance, the radiations are powerfully reflected backwards by the crystal. This is the result of the optical vibrations of the crystal lattice being strongly excited by the incident waves. That such an excitation results in a directed reflection and not a diffuse scattering clearly indicates that the vibration of the crystal lattice is in the same phase at every point on the surface of the crystal. In the more general case of oblique incidence, in order that a regular geometric reflection may result, the excited lattice vibrations have necessarily to be coherent in phase, having everywhere the same relation to the phase of the resultant electric force due to the superposition of the incident and reflected waves. Since the waves necessarily penetrate to a certain depth, it follows that such coherence in phase must extend also into the interior of the crystal.

Passing on to the case of the scattering of light within a crystal, it is evident that an optical vibration of the crystal lattice cannot be excited by the incident light if the phase of such vibration is the same throughout the volume of the crystal. This is evident from the principle of interference, as the effects of all the secondary radiations, irrespective of their frequency, would completely cancel out. In fact, it is easy to show that a scattering of light with altered frequency within a crystal would only be possible if the lattice-vibrations have phase waves which are equally inclined to the incident and scattered waves and so spaced that the scattering is in effect a monochromatic reflection by an optically stratified medium. A very similar situation also arises when we consider the scattering of light as the result of the acoustic vibrations of the crystal lattice. The usual formula for a monochromatic reflection connects the wave-length of the incident radiation, the spacing of the optical stratifications and the glancing angle which is half the angle of scattering. This formula which follows from the classical wave-principles has its counterpart in the quantum theory, appearing as a consequence of the conservation of energy and momentum in the collision between the photon and the acoustic or optical disturbance in the crystal.

Having considered the cases of infra-red radiation, and of ordinary light, we naturally pass over to the X-ray optics of crystals. It is fairly obvious that, as in the case of ordinary light, an acoustic vibration of the lattice can only give rise to a diffuse scattering of the X-rays. The position is entirely different in regard to the optical vibrations of the crystal lattice. It follows from the very nature of an optical vibration that it does not involve any variation in the mean electronic density of a unit cell in the lattice,

but can cause only periodic variations of the structure-amplitudes of the crystal. In other words, the effect of an optical vibration is to cause a variation having its own frequency in the reflecting power of the regular crystal spacings. We may put this a little differently by stating that the optical vibration creates dynamic stratifications of electron density, and that these can give regular X-ray reflections but with a change of frequency, in much the same way as the static planes give the classical reflections without change of frequency. The spacing and orientation of the dynamic stratifications are identical with those of the static spacings when the phase-wave-length is infinite. More generally, these quantities and, therefore, also the geometric law of the modified reflection, would depend on the wave-length of the phase-waves and the angles which they make with the crystal spacings and with the plane of incidence. The geometric law of quantum X-ray reflection for the most general case has been deduced by Raman and Nath in a paper published in the *Proceedings* of the Academy for November 1940, and takes a quite simple form. It will suffice here to remark that the theory shows that the reflection should appear in precisely specifiable directions. In other words, *the spacings of a crystal should give, in addition to the classical or unmodified reflections, quantum or modified reflections obeying a different geometric law.* It is no exaggeration to remark that this new result is of the greatest importance both to X-ray optics and to crystal physics. For the experimental proof of this thesis, it is necessary that sharply defined X-ray reflections of which the positions agree with those theoretically deduced should be found in association with every crystal plane of which the structure amplitude is sufficiently large and is strongly modulated by the possible vibrations of the lattice. That this is actually the case has been demonstrated in a series of communications published during the year by Raman and Nilakantan. A specially detailed investigation for the case of diamond is appearing shortly in the *Proceedings*, which brings out in a convincing way the physical reality of the phase-waves associated with the optical vibrations of the crystal lattice. The prediction made earlier in the year that the intensity of the modified reflections given by diamond should remain unaffected at liquid air temperatures has also been completely confirmed. *The quantum theoretical character of the new reflections has thus been completely established.*

Returning to the case of the scattering of ordinary light, it may be remarked that both the acoustic and optical vibrations of the crystal lattice reveal themselves in it. The vibrations of the latter class are usually subdivided into external and internal vibrations. Though this distinction is somewhat arbitrary, it is in many cases useful; and indeed, in relation to the crystal structure, the external vibrations, the frequency of which is determined by the crystal fields and are usually much lower, are even more important

than the internal vibrations. The thermal behaviour of the external oscillations is particularly interesting. The very careful studies of the temperature effect which have been made by Nedungadi in the cases of sodium nitrate and of quartz have been very illuminating. Nedungadi's studies indicate that any transformation in crystalline form is usually *preceded* by notable changes in the magnitude and character of the low-frequency shifts. The changes observed in the case of α-quartz greatly assist in understanding the remarkable variations of physical properties which precede the α–β transformation of quartz.

The case of α-quartz has been very exhaustively investigated by Saksena with a view to identifying the various modes of optical vibrations appearing in the spectrum of the scattered light and correlating these with the known infra-red spectrum of α-quartz and with the specific heat of the crystal. The investigation must be considered to have been highly successful, as the theoretically predicted and experimentally observed behaviours show an almost complete agreement. The theoretical part of the investigation was based on an application of the methods of the group theory to the known structure of the crystal and the determination of its symmetry modes of vibration. The verification of the theoretical conclusions required an investigation of the spectra with the incident light polarised in different ways and with the crystal in different orientations, as also an analysis of the scattered light in each case. Such an investigation is naturally laborious and time-consuming, but the results in the case of quartz appear fully to have justified the trouble taken. It is very satisfactory to find from the work of Bhagavantam with calcite, and of Saksena with quartz, that the theoretical selection rules for the appearance and non-appearance of certain vibrations in the spectra are found to be obeyed. These selection rules may be deduced geometrically from the known form of the optical polarisability ellipsoid of the crystal and the character of the deformations it should undergo for vibrations of the different possible types of symmetry. The fact that the results deduced are in accord with the experimental results shows clearly that we are dealing with the vibrations of the crystal lattice and not of the individual chemical units in it, as remarked earlier in this address.

Summary

The excitation of the optical modes of vibration of a crystal lattice by radiations incident on the crystal is discussed. The optical vibrations can be described as oscillations of the interpenetrating lattices in the crystal with respect to one another. They give rise to a periodic variation of the physical constants, *e.g.*, electric dipole-moment, optical polarisabilities, structure amplitudes, which influence the behaviour of radiation in various ranges of frequency in its passage through the crystal. The phenomena observed in

the different ranges of frequency have a common feature, namely that the incident radiation excites the crystal vibrations of which the phases are everywhere in coherent relationship with the phase of the radiation field. The scattering of light or the reflection of X-rays with change of frequency appears as the result of the phase of the lattice vibrations varying from point to point in such manner that the crystal is, in effect, an optically stratified medium giving a monochromatic reflection of the incident rays at the appropriate angle of incidence determined by the spacing of the stratifications and the wave-length of the incident radiation. The description of the observed effects in the language of the wave-theory and in terms of the quantum hypothesis are complementary and do not in any way contradict each other.

The paper includes a review of 14 communications dealing with this field of research published during the year 1940 in the *Proceedings of the Indian Academy of Sciences.*

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10. B. D. Saksena .. "Raman Effect and Crystal Symmetry."
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AN AUTOTRIPLOID IN THE PEARL MILLET (*PENNISETUM TYPHOIDES* S. & H.!)

By N. Krishnaswamy, B.Sc., Ph.D.
AND
G. N. Rangaswami Ayyangar, F.N.I., I.A.S.
(*Millets Breeding Station, Agricultural Research Institute, Coimbatore*)

Received October 3, 1940

1. *Introduction*

Triploids have been recorded amongst the cereals in Wheat (Thompson, 1929; Mather, 1935), Maize (Randolph and McClintock, 1926; McClintock, 1929), Rice (Nakamori, 1932; Ramiah *et al.*, 1933; Ramanujam, 1937), Oats, Barley and Rye (Müntzing, 1938). So far the occurrence of a triploid has not been reported in any of the millets and the present observation thus forms the first record of a triploid in *Pennisetum typhoides*.

Origin of the Triploid.—The present triploid is the progeny of a sterile plant which was observed in the lot M.S. 4477—*Arupatham Cumbu*, Velur, a fresh arrival at the Millets Breeding Station, Coimbatore, and grown in the summer of 1939. This sterile plant was about 100 cm. high with a number of tillers. The stems were thin and leaves normal; earheads rather short (15·9 cm.). The plant though surrounded by normal diploids and itself produced enough, free pollen failed to set seed. On examination the pollen did not show any indication of high sterility. Since the plant is protogynous and there were no seeds set in spite of abundance of pollen, it was thought to be female-sterile. The plant was noted rather late in the season and therefore efforts were made only to secure some seeds from it and no materials gathered for cytological examination because of the paucity of earheads. The stigmas were repeatedly dusted with pollen from other heads from the same plant and nine seeds were obtained from about a dozen heads. These seeds therefore were in a manner, from selfed heads.

The seeds were sown in pots in summer 1940 and later transplanted into the field. Only seven seeds germinated. Out of these seven plants four proved to be normal diploid, about 250 cm. high with full setting of seeds. Of the remaining three one was a partially sterile dwarf 38 cm. high with thin stems and produced only one head. This plant gave the diploid number of $2n = 14$ in the root-tips. The second plant was also partially sterile but the plant was vigorous and the seeds set fairly well.

The third plant was vigorous and healthy; the leaves were normal. Vegetatively the plant showed no difference from the others. Towards flowering time, however, the sheath showed a swelling above the flag node,

FIG. 1 (*a*)
(*a*) Diploid plant. 1/20 natural size

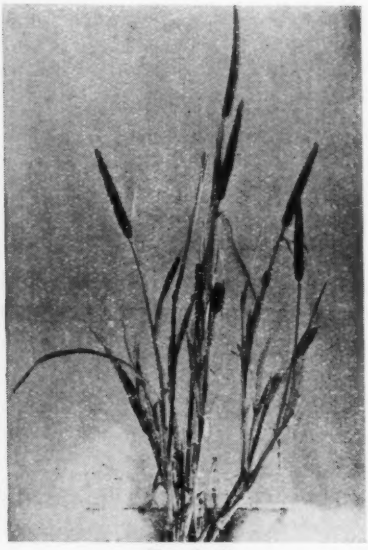

FIG. 1 (*b*)
(*b*) Triploid plant. 1/12 natural size

but no panicles emerged. On opening this portion a short highly abnormal panicle was seen. The spikelets were short, malformed and often much thickened. The glumes had a membranous, ligulate outgrowth at the tip. The stigmas and anthers were found in various stages of suppression. Some of the flowers were seen to have yellowish green, normally developed anthers.

The later heads of this plant were freely emerging and normally developed (Fig. 1 *b*). This plant on examination proved to be a triploid. It, however, showed no difference in other respects from the diploid. The following gives a comparative idea of pollen size, etc., between the diploid and the triploid:

	Height of plant	Length of leaf	Length of anther	Stomata		Pollen diameter	Sterility
	cm.	cm.	mm.	Length μ	Breadth μ	μ	%
Diploid ..	250	50	2·6	33·6	27·1	38·8	8
Triploid ..	100	38	2·5	34·4	30·7	32·9	50

2. *Cytology*

Materials and Methods.—The root-tips of the diploid and the triploid were fixed in Levitsky's fluid. The flower buds were gathered in the triploid both from the malformed and the well-formed, freely emerging earheads and fixed in modified Karpechenko's fluid. The sections were stained according to Feulgen's technique and counterstained with Fast-green (Jacob). The drawings were done with a Spencer's Camera-Lucida and Zeiss microscope. Unless otherwise stated all figures are drawn to a magnification of about × 1500.

(*a*) *Mitosis*—

The counterstaining with Fast-green brought the nucleoli into prominence so that it was possible to distinguish in early telophase in the triploid three small nucleoli initiating themselves each on one chromosome (Fig. 2 *f*). In the resting nucleus three nucleoli could be counted and in no case were

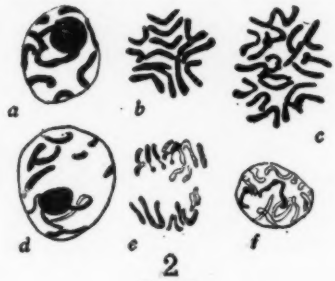

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FIG. 2

(*a*) Diploid, prophase showing nucleus with 2 nucleolar chromosomes. (*b*) Metaphase plate of diploid with 14 chromosomes. (*c*) Triploid metaphase plate—21 chromosomes. (*d*) Triploid prophase with 3 nucleolar chromosomes. (*e*) Telophase of triploid showing 3 sat-chromosomes. (*f*) Early telophase of triploid showing the 3 nucleoli initiating on nucleolar chromosomes.

there more than three, while owing to fusion of the three nucleoli numbers less than three were met with, *i.e.*, two or one. In such cases one nucelolus was always seen to be smaller than the other, showing that the larger has arisen from the fusion of two nucleoli. Fig. 2 *d* shows the nucleolus at the prochromosome stage with three chromosomes attached to one large nucleolus. At the prophase it was not possible to definitely find out whether all the three nucleolar chromosomes had satellites. However, in the side-view of a telophase three sat-chromosomes were seen (Fig. 2 *e*).

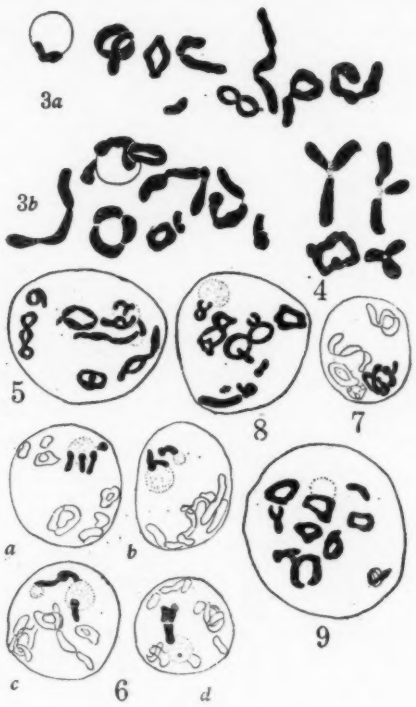
Metaphase.—At metaphase the chromosomes are rather long and hence not lying in one plane (Fig. 2 *c*). In some cases the three nucleoli persist at metaphase attached to their chromosomes. Sat-chromosomes are seen in metaphase but all three could not be seen in one and the same plate. The number of chromosomes as counted in the metaphase plate was found to be twenty-one. These metaphase chromosomes were not sufficiently distinctive to recognise homologues of each set.

The chromosomes of the diploid *Pennisetum* have been studied by Rau (1929), Audulow (1931), Rangaswami (1935) and Krishnaswamy (1939). The root-tip of a diploid, sister plant to the triploid, was examined for comparison. At prophase the maximum number of nucleoli produced were found to be two and only two chromosomes were seen attached to the nucelolus (Fig. 2 *a*). The number of chromosomes counted at the metaphase was fourteen (Fig. 2 *b*). The diploid to a certain extent showed somatic pairing and the homologues could be somewhat distinguished.

(*b*) *Meiosis*—

The prophase stages were difficult to study on account of the thinness of the threads. The stages were therefore followed from diakinesis onwards.

Diakinesis.—At diakinesis the chromosomes are much thickened and shortened. Trivalent associations are common. The most frequent configurations are chains of three Y's, and the ' Frying-pan ' types. In three cases a ring of three chromosomes was observed. The trivalents usually occurred with a few bivalents and univalents. The most common associations are five and six trivalents with varying numbers of bivalents and univalents. Figs. 3 *a* and *b* represent two P.M.C.'s at diakinesis, with the chromosomes spaced apart. Fig. 3 *a* shows $4_{III} + 4_{II} + 1_{I}$, and Fig. 3 *b*—$4_{III} + 3_{II} + 3_{I}$. In Fig. 3 *b* is seen also a ring trivalent with terminalised chiasmata. Fig. 4 shows two Y-trivalents, one ring with non-terminalised chiasmata and one with triple chiasma. Fig. 5 is at late diplotene showing $2_{III} + 7_{II} + 1_{I}$. In only four cases seven trivalents, the maximum number possible, were observed.



FIGS. 3–9

Fig. 3.—Diakinesis. × 2250. Fig. 4.—Diakinesis, figures Y-shaped, ring of three and triple chiasmata. × 2250. Fig. 5.—Late diplotene. Fig. 6.—Pairing of nucleolar chromosomes. Fig. 7.—Interlocking. Fig. 8.—Late diplotene abnormal pairing. Fig. 9.—Diakinesis, showing no trivalents.

The nucleolar chromosomes usually form a bivalent and a univalent (Fig. 6 *b*). The univalent is always attached to a small nucleolus while the bivalent to a single large one. In Fig. 6 *c* two of the nucleolar chromosomes have paired at the free ends. At the other end they are attached one to a large nucleolus to which also a univalent is attached and the other of the pair to a smaller nucleolus. In these only the portions of the chromosomes free of the nucleolus are seen paired and never the region attached to the nucleolus. The only type of configuration these chromosomes form is the Y-type. The nucleoli interfere in the free association of the chromosomes and in one case all the three were seen side by side attached to one large nucleolus (Fig. 6 *a*). Owing to this mechanical interference the

nucleolar chromosomes almost always contribute to the number of the univalents. The following gives the frequency of the trivalent formations :

									Total
Trivalents per P.M.C. ..	..	0	1	2	3	4	5	6	7	
Number of P.M.C.'s observed	..	2	1	..	3	12	25	19	4	66

Mean number of trivalents per cell = 4·95. In the two cases in which a complete failure of trivalent formation was noted there were $8_{II} + 5_I$ and $10_{II} + 1_I$ (Fig. 9).

A few abnormalities in pairing were observed. Fig. 8 shows three ordinary bivalents, two figure of eight configurations and some univalents. One of the univalents is abnormally large while the other is normal. One P.M.C. showed two ring bivalents interlocked (Fig. 7). Associations of more than three chromosomes are drawn in Fig. 10 (*a*—shows a ring of three and a rod; *b*—shows a frying-pan type with two chromosomes attached in

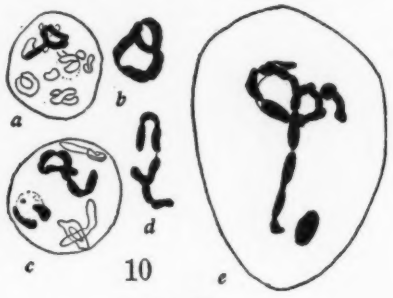


FIG. 10

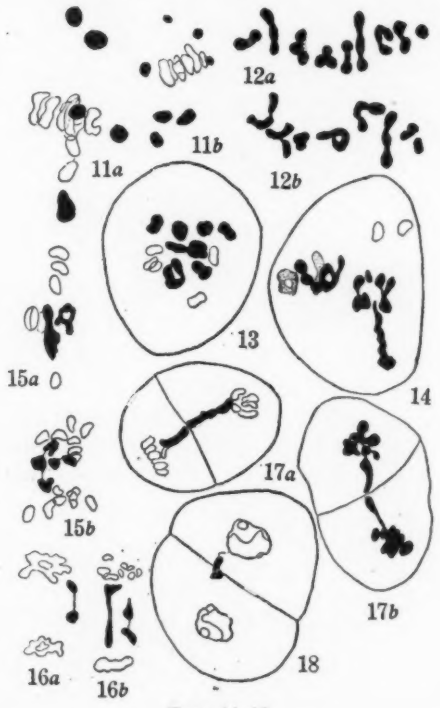
Multivalent associations at diakinesis: (*a*) A ring of three and a rod. (*b*) Chain of five. (*c*) and (*d*) Chain of 7. (*e*) Chain of 10.

tandem to the rod; *c*—is a chain of seven; *d*—consists of a normal chain of three associated to a Y-type, while one of the arms of the Y has another chromosome; *e*—is a chain of ten chromosomes *plus* one chain of three + $1_{II} + 1_I$).

Metaphase.—At metaphase the chromosomes form a more or less loose equatorial plate. The trivalents, bivalents and some univalents are arranged in a haphazard manner. The univalents are found mostly distributed in the spindle well outside the equatorial plate. Two metaphase plates are shown in Figs. 11 *a* and *b* with five and seven univalents. In Fig. 12 *a* and *b* are drawn two metaphase plates spaced out (*a*) $5_{III} + 2_{II} + 2_I$; (*b*) $6_{III} + 1_{II} + 1_I$. Fig. 13 is a polar view containing $2_{III} + 5_{II} + 5_I$. The number of univalents

occurring at the first metaphase plate varied from zero to seven, the most common number being one, two or three. A normal bipolar spindle is formed.

Anaphase.—At anaphase the bivalents are seen to separate first. The trivalents disjoin in two and one each going to one pole. In Fig. 14 at early anaphase ($5_{III} + 2_{II} + 2_I$) three trivalents are seen disjoining into two and one. In Fig. 15 *a* is a ring trivalent with interstitial chiasma and a chain trivalent separating into one and two. Fig. 15 *b* shows a trivalent rather late in disjoining. Very often one to two trivalents were seen lagging owing to delay in disjunction (Figs. 16 *a* and *b*). Chromatin bridges were observed in a few cases. These bridges persist so long that they get cut in two by the



FIGS. 11–18

Fig. 11.—Side view of metaphase I; (*a*) × 2250,—showing 5_I; (*b*) showing 7_I. Fig. 12. metaphase I. Side view chromosomes spaced out: (*a*) $5_{III} + 2_{II} + 2_I$; (*b*) $6_{III} + 1_{II} + 1_I$. Fig. 13.—Polar view of metaphase I. Fig. 14.—Anaphase early. Fig. 15.—Anaphase; disjunction of the trivalents. Fig. 16.—Lagging of bivalents and trivalents. Fig. 17.—Chromatin bridge first division, cut by the interphase wall. Fig. 18.—Fragment caught in the interphase wall with remnant of bridge.

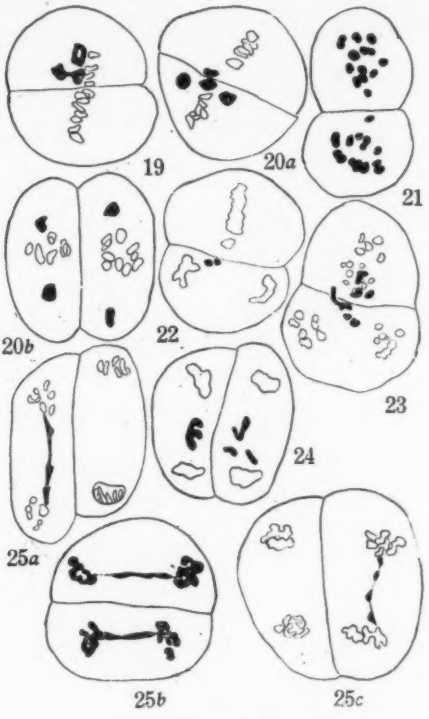
growing interphase wall (Figs. 17 *a* and *b*). In a few instances one to two univalents were found dividing at the equator towards late anaphase. It was frequently observed that the univalents after coming to the equator failed to divide and move to the poles, consequently they were also cut in two by the growth of the interphase wall. The fragmentation of the chromosomes thus resulted. The analysis of thirteen clear late anaphase stages gave the following distributions of the chromosomes. In a few of them it was seen that trivalents had moved to the poles without disjunction.

Distribution of the chromosomes at first early telophase

12	1	8	1	
11	0	10	2	
11	1	9	1	
11	2	8	4	(Two trivalents without disjunction)
10	1	10	1	
10	2	9	1	
10	3	8	1	
10	1_{III}	8	1	(One trivalent lagging)
9	4	8	1	

Telophase.—A regular interphase nucleus with nucleoli is formed. A few cells show the persistent first division bridge attached to a fragment (Fig. 18). Sometimes the fragment is cut in two by the cell wall. This particular fragment showed a subterminal achromatic portion. Univalents are sometimes seen lying near the poles without moving. These get included in the first division daughter nuclei.

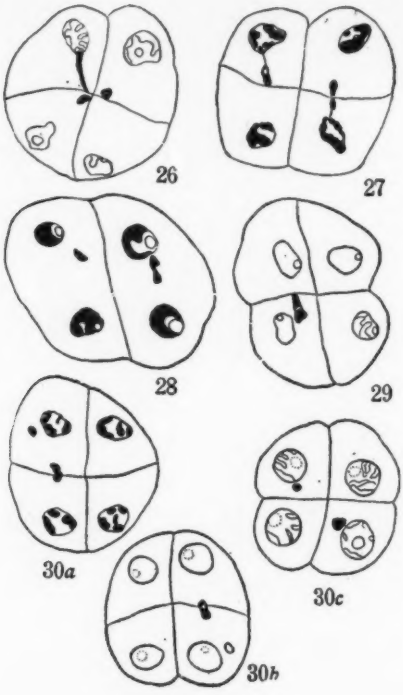
Second Division.—The second division plates are normal, but very often they are loosely formed (Fig. 19). Univalents of varying numbers are found distributed on the spindle. Some of them are at the poles while the others are still at the equatorial plate (Figs. 20 *a* and *b*). Polar view of second metaphase often shows chromosomes in excess of the expected numbers. This is probably due to univalents dividing and getting inclosed in the same nucleus (Fig. 21) (Randolph and McClintock, 1926; Kostoff and Kendall, 1931), the most common distribution being 9–11 with a univalent lagging. The exact numbers could not be determined in many second metaphase plates, especially where the numbers were high, owing to secondary associations and also due to the divided chromosomes sticking close together. Usually both cells divide together but in some cases one daughter cell may



FIGS. 19–25

Fig. 19.—Metaphase II showing 2_{II} in one plate. Fig. 20.—Univalents at metaphase II. Fig. 21.—Polar view of metaphase II. Fig. 22.—Disparity in the division time of the daughter cells. Fig. 23.—Univalents left on plate in division II. Fig. 24.—Trivalents disjoining at second division. Fig. 25.—Second division bridges.

be in telophase while its sister cell is still at metaphase (Fig. 22). In such cases usually the nuclei with smaller chromosome numbers divide earlier. In one P.M.C. a bivalent and a trivalent were found lagging each in one daughter nucleus (Fig. 24). Chromatin bridges are more frequent in the second division than in the first division. Either one daughter cell (Fig. 25 *a-e*) or both (Fig. 2 *b*) show the bridges. Tetrads are regularly formed and no restitution nuclei are formed. However, in many tetrads (Figs. 28–30) fragments and univalents occur in the cytoplasm. Fig. 23 shows univalents remaining at the equatorial plate at telophase. Figs. 26 and 27 show fragments in tetrads with chromatin bridge still persisting.



FIGS. 26–30

Fig. 26.—Remnant of bridge and fragment cut by the intersecting wall. Fig. 27.—Two fragments one in each cell. Fig. 28.—A univalent and a fragment in the cytoplasm. Fig. 29.—A large fragment with achromatic portion. Fig. 30.—Fragments in tetrads.

3. *Discussion*

The frequency of the formation of the trivalents in this plant is high (5 per P.M.C.) and the maximum number of trivalents that can be formed in a P.M.C., *i.e.*, seven, was observed in 6 per cent. of the cells counted. The trivalents are usually found along with bivalents and univalents. Müntzing (1936) remarks that in experimental autopolyploids having more than two homologous chromosomes of each kind the formation of multiple associations is very characteristic. The maximum number of chromosomes in these associations correspond to the number of genomes present. He concludes: "In short the presence of multivalents indicates autopolyploidy and the absence of allopolyploidy." The occurrence of bivalents and univalents has been noted in other autotriploids also (Bleier, 1934). In fact, the maximal theoretically possible chromosome association never occurs

in reality. The chromosome association is almost always incomplete and this incompleteness varies considerably in different species (Müntzing, *loc. cit.*). The causes for failure of pairing are several. Kostoff and Kendall (*loc. cit.*) refer the varying number of trivalent formation to the degree of affinity among the homologous chromosomes. Sapehin (1933) is of the opinion that the conjugation of chromosomes is conditioned by genes and that the external conditions also exert a great influence on the conjugation of chromosomes. Federly (1932) is of a similar opinion. Dark (1932) considers the competition in pairing between the three homologous chromosomes of the triploid as the real reason for the failure of pairing. Darlington and Mather (1932) point out that if association between two members of a set of three homologous chromosomes were very extensive the chances of a chiasma forming between the free portion of one and the third chromosome would be correspondingly reduced and a bivalent and a univalent would result. Darlington (1929), however, says that failure in a polyploid to form trivalents, quadrivalents and so forth at the metaphase can in some measure be attributed to inability of a chromosome to maintain a connection with more than one other at a time and not necessarily to lack of affinity for more than one of its homologues. Further according to Müntzing (*loc. cit.*) non-conjugation does not necessarily mean non-homology, but conjugation is a strong indication of homology. Sansome and Philp (1939) distinguish triploids resulting from crossing two plants of different phylogeny and those arising through crossing of plants of similar origin. The first having three sets of chromosomes of which at least one is distinct from the other two is called allotriploid. The second class with all three sets of chromosomes homologous is called autotriploid. These evidences support the view that the plant described is an autotriploid. Its constitution may be represented as

A B C D E F G
A B C D E F G
A B C D E F G

The trivalent configurations are of the types expected in an autotriploid (Darlington,[1] 1937). The ring of three chromosomes, however, is an exception. This type of trivalent formation has been observed by other authors also (Affify, 1933; Philp and Huskins, 1931). Affify derives the ring of three chromosomes by assuming two homologous ends in one of the chromosomes (owing to segmental interchange). Thus the three chromosomes would be A–B, A–B and A–A, which would form a ring in diakinesis.

Multiple associations of more than three chromosomes have been met with in other triploids,—in *Aconitum* (Affify, *loc. cit.*), Rice (Ramanujam,

loc. cit.), *Mathiola incana* (Philp and Huskins, *loc. cit.*). These configurations are considered to be due to segmental interchange in some of the chromosomes. The complete failure of the trivalent leads to the formation of bivalents as in the case of the 9 and 10 bivalents. Yarnell (1929) in *Fragaria* ascribes these to pairing in non-homologous chromosomes. Darlington[1] (*loc. cit.*) however is of the opinion that these are all owing to interchange in the chromosomes.

Lagging and bridge-formation have been observed in the first and with a greater frequency in the second division. In some of the P.M.C.'s both daughter nuclei showed each one bridge. One fragment in particular, with an achromatic portion, has been observed both at the interkinesis and also at the second division. The behaviour of this fragment indicates that it is acentric. Bridge-formation is the result of inversion. The presence of bridges at the first anaphase as also in the second indicates that crossing-over has occurred between two dislocated chromatids giving a dicentric chromatid forming a bridge at the first division. The pairing of dislocated segments within one chromosome (inverted duplication) may occur as part of a trivalent combination. The two centromeres in such cases may pass to the same pole but a reciprocal bridge will form at the second division anaphase (Darlington,[1] *loc. cit.*). Westfall (1940) has shown that inversions in one of the homologues of a set of three may lead to bridge formation either in the first or the second anaphase or no bridge formation at all depending on the random assortment of the dicentric chromosomes, but almost always an acentric fragment would result. Emsweller and Jones (1937) also showed fragmentation without bridge formation and put forward the probability of bridge formation without inversion. This they show is possible " when two chromosomes pair in such a manner that their insertion regions are not opposite each other and a single cross-over occurs in the interval between the insertions, a bridge will ordinarily result ". They have found this type of pairing common in *A. cepa* × *fistulosum* hybrids. The present case is considered to be like those described by Westfall (*loc. cit.*) where inversion has occurred in one of the three homologous chromosomes of a trivalent and the bridge formation in the first or the second division being dependent on the random assortment of the dicentric chromosome.

The triploid may arise in hybrids between a tetraploid mother and a diploid father or as a mutation in a diploid population. Müntzing[1] (*loc. cit.*) has described a number of cases in which the triploid has arisen as one of the twin plants. The triploid can arise in a diploid population either (1) by dispermy, *i.e.*, two sperms fertilising the same egg (Rhoades, 1936; Ramanujam, *loc. cit.*), or (2) a diploid gamete may unite with a haploid

gamete. The diploid gamete may be the sperm (Rhoades, *loc. cit.*) or the egg. The latter is more common since a diploid egg is more functionable than a diploid sperm. Watkins (1932) has shown that lowering in the normal ($2n$ mother tissue and n pollen) ratio of the pollen tube to the female parent tissue in respect of their chromosome numbers results in the retardation of the growth of the pollen tube and fertilisation fails. Huskins (1934) records the possibilities of the triploid arising somatically. The present triploid has obviously arisen by the fusion of diploid and haploid gametes. It could not be said which of the gametes was diploid.

The plant produces some free pollen but yet it is highly sterile. A few seeds have been obtained by pollinating with the pollen from other heads of the same plant. A number of ears are being dusted with pollen from diploid plants. Levan (1936) found in the somatic counts of the progeny reciprocal crosses between diploid and triploid *A. schænoprasum* considerable differences in number according to whether the $2n$ or the $3n$ was the female parent. With $2n$ as the mother mostly diploids were obtained, while with $3n$ as mother all numbers from $2n$ to $3n$ were obtained. Huskins (*loc. cit.*) curiously enough obtained only $4n$ plants in the progeny of the triploid tomato. $4n$ Plants have also been reported in the progeny of other triploid plants (Sansome and Philp, *loc. cit.*).

4. *Summary*

(1) An autotriploid plant was noted in the progeny of a sterile plant in *Pennisetum typhoides*, S and H.

(2) Vegetative characteristics of this plant did not differ in any way from the diploid.

(3) The $2n$ number of the plant is 21. The diploid shows $2n = 14$.

(4) The meiosis showed a high frequency of trivalent formation. The most common configurations were chains of three, frying-pan and Y-types. Rings of three chromosomes were noted in a few cases.

(5) Higher associations than three were met with. These are considered to be due to segmental interchange.

(6) Fragmentation and bridge formation were frequent at the first and second anaphases. Inversion has taken place in one of the homologues of a trivalent. This plant therefore belongs to the structural hybrids class.

(7) Tetrads were normally formed and some free pollen is obtained.

(8) The plant is highly sterile. It is being crossed with diploid plants and also self-pollinated with a view to study the progeny.

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MORPHOLOGICAL AND CYTOLOGICAL STUDIES IN THE SCROPHULARIACEÆ

III. A Contribution to the Life-History of *Ilysanthes parviflora* Benth.

By Dr. T. S. Raghavan, M.A., Ph.D. (Lond.), F.L.S.
(*Head of the Department of Botany, Annamalai University*)
AND
V. K. Srinivasan, M.Sc.
(*Annamalai University*)

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I. Introduction

The first two papers of this series (Raghavan and Srinivasan, 1940; and Srinivasan, 1940) described the cytology of *Angelonia grandiflora*, and gave the chromosome numbers of about nine species distributed over four genera, as also the development of the anther, the embryo-sac, endosperm-haustorium and the embryo in *Angelonia grandiflora*, *Dopatrium lobelioides*, *Stemodia viscosa* and *Vandellia crustacea*. The genus *Ilysanthes* has not received any attention except for a small note by Krishna Iyengar (1929) on the development of the embryo-sac and endosperm-haustoria in an undetermined species of *Ilysanthes*. And the chromosome number of not even a single species of *Ilysanthes* has been determined so far. The haploid chromosome number of *Ilysanthes parviflora* has been recorded for the first time in the present communication. A detailed account of the ontogeny of the male and female gametophytes and of the embryo is given. The origin

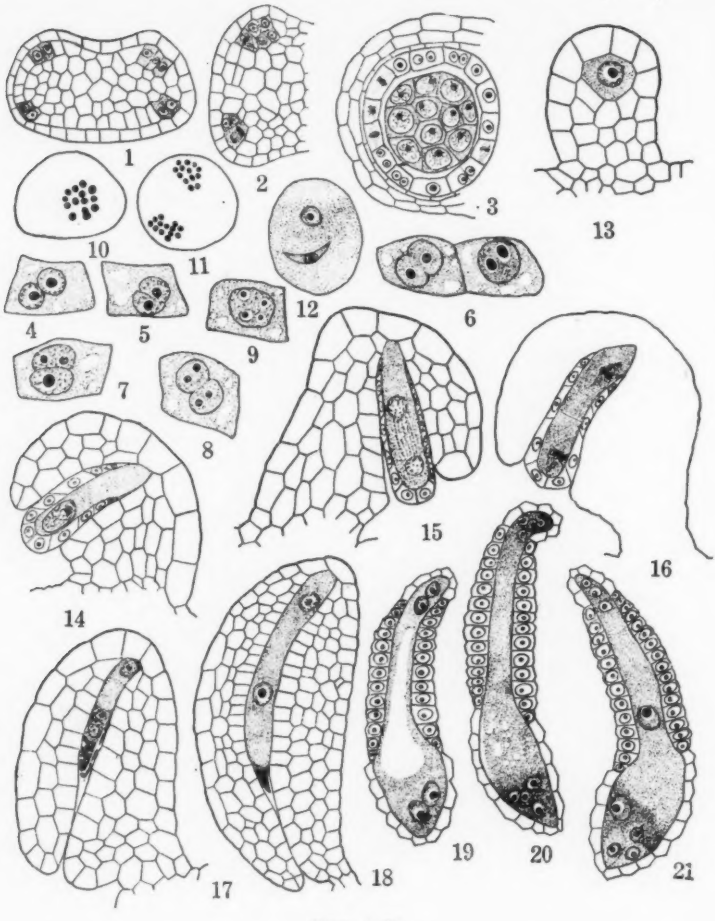
and development of the endosperm-haustorium is also described in detail. *Ilysanthes parviflora* is an erect branching herb. Flowers are borne in short racemes in the axils of leaves or at the ends of branches. The corolla which is bilobed is white in colour. There are two fertile and two undeveloped stamens.

II. Material and Methods

A few plants were found growing near rice-fields in Manalur, from which material for the present work was obtained. Only a few plants were available and as such sufficient anthers could not be got for any acetocarmine examination. Moreover, the anthers were so small that individual fixation was not advisable. Whole buds were therefore fixed in hot corrosive-sublimate fixative. Formalin-Acetic-Alcohol proved worthless for the purpose. Ovaries of various stages of development were fixed either in hot corrosive-sublimate or in Formalin-Acetic-Alcohol. Sections were cut at thicknesses varying from 6–14 microns and stained in Haidenhain's Iron-alum-hæmatoxylin.

III. Observations

(*a*) *Microsporogenesis.*—The primary archesporium of the anther lobe consists of a row of two or three hypodermal cells (Fig. 1) and is differentiated soon after the anther appears four-lobed in cross-section (Fig. 1). The archesporial cells are more prominent than the surrounding cells. The anther lobes become more and more prominent after the differentiation of the primary archesporium. The hypodermal archesporial cells cut off a layer of primary parietal cells (Fig. 2). The primary wall cells divide periclinally repeatedly giving rise to three layers of wall cells, the innermost of which functions as the tapetum (Fig. 3). The layer of wall cells immediately outside the tapetal cells get elongated tangentially and is finally crushed. The tapetal cells enlarge in size and small vacuoles make their appearance (Fig. 4). They are to begin with uni-nucleate. The single nucleus of the tapetal cells undergoes a division, just at the time, when the pollen mother-cells are in early prophase. This division is mitotic in nature. Fig. 3 shows various stages in the mitotic division of the tapetal nucleus. The further behaviour of the two nuclei of the tapetal cells is rather irregular. Sometimes, the two nuclei fuse to form a single large bi-nucleolated nucleus (Fig. 5). Fig. 6 shows two adjoining cells, in one of which the two nuclei are in a process of fusion, while the other shows the fused product. Often, however, the two nuclei divide again, the resulting four nuclei fusing immediately. This fusion follows so quickly upon the division, that it is hard to find them in a separate condition. Figs. 7 to 9 show the nuclei in various processes of fusion. In the nucleus which is thus organised, can be seen the four



FIGS. 1–21

FIG. 1.—T. S. young anther, showing hypodermal archesporial cells. × 700. FIG. 2.—Archesporium cutting off wall cells. × 700. Fig. 3.—Shows the three layers of wall cells and the mitotic division of tapetal nuclei. × 700. FIG. 4.—Binucleate tapetal cell. × 2225. FIGS. 5–9.—Tapetal cells showing the nuclei in various processes of fusion. FIG. 10.—Metaphase I showing 13 bivalents. × 2225. FIG. 11.—Metaphase II, shows two groups of 13 univalents each. × 2225. FIG. 12.—Two-celled pollen grain at the time of shedding. × 1500. Fig. 13.—Hypodermal archesporium in the ovule. × 1500. FIG. 14.—Megaspore mother-cell invested by a single layer of nucellar cells. × 1500. FIG. 15.—Heterotypic division of the mother-cell. × 1500. FIG. 16.—Division of the dyad. × 1500. FIG. 17.—Linear tetrad. × 1500. FIGS. 18–21.—Two, four, and eight-nucleate embryo-sacs. Note the tapetum, which surrounds only the non-dilated chalazal portion. In FIG. 21.— the chalazal polar nucleus has reached the centre of the embryo-sac earlier than that of the micropylar end. × 1200.

nucleoli (Fig. 9). Very often, these various stages are represented side by side in the same tapetum. A similar behaviour of the tapetal nuclei has been recorded in *Gynandropsis pentaphylla* (Raghavan, 1938). The fused tapetal nucleus at pollen grain stage begins to disintegrate.

In metaphase, I, 13 bivalents are present in the equatorial plate (Fig. 10). Meiosis appears to be regular and disjunction is normal, there being no lagging chromosomes. Consequently, in metaphase II, the usual 13/13 distribution is the rule. Fig. 11 shows such a metaphase II showing the polar view of the two plates. Pollen tetrads are organised in the normal manner. Fig. 12 shows a pollen grain just at the time of shedding. The crescent shaped cell is the generative cell.

(*b*) *Megasporogenesis.*—The ovary is typically scrophularious being bicarpellary, and having numerous anatropous ovules arranged on an axile placenta. Early in the development of the ovule, the hypodermal archesporial cell is differentiated (Fig. 13). This takes place even before the integumental primordia have been initiated. This is a characteristic feature of not only the scrophulariaceæ but also of many other families. This archesporial cell directly functions as the megaspore mother-cell (Fig. 14) without cutting off any wall cell. By this time, the single massive integument grows rapidly accompanied by the anatropous curvature of the ovule (Fig. 15). The bulk of the ovule at this stage is made up of the integumentary tissue (Fig. 15). The megaspore mother-cell is invested almost up to its base by a single layer of nucellar cells (Fig. 14). This nucellar layer is derived from the original domed apex, which caps the archesporial cell. The megaspore mother-cell elongates considerably, after which it undergoes the heterotypic division forming a dyad (Fig. 16). The dyad by an ordinary homotypic division produces a linear tetrad of megaspores (Fig. 17). During the formation of the linear tetrad, the single layer of nucellus gets pressed against the integument and begins to degenerate, its place being taken up by the innermost layer of cells of the integument. This layer of cells is known as the tapetum, which is thus integumentary in its nature. It is composed of a regularly arranged layer of cells, which assume ultimately a more or less radially elongated configuration. The cells of this integumentary tapetum take deeper stain than the other cells of the integument and are uni-nucleate. A similar tapetal tissue of integumentary origin is characteristic of most members of the scrophulariaceæ. While the cells of this tapetum however, are all uni-nucleate, in families like Solanaceæ (Bhaduri, 1932) and Orobanchaceæ (Srivastava, 1939), binucleate tapetal cells would appear to be the rule.

FIGS. 22–36

FIG. 22.—Mature embryo-sac showing the egg-apparatus and the small-sized antipodals. × 1200. FIG. 23.—Note the male nucleus about to fuse with the egg, as also the remains of the pollen tube and synergids. × 1200. FIG. 24.—Two endospermal cells. × 1200. FIG. 25.—Shows the two uni-nucleate chalazal haustorial cells and micropylar chamber composed of two tiers of two cells each. × 1200. FIG. 26.—A micropylar tier of four cells has been cut off from the middle tier composed of four cells. × 1200. FIG. 27.—Shows the two uni-nucleate chalazal haustoria, four-nucleate micropylar haustorium and the elongated oospore, prior to division × 750. FIGS. 28-35.—Various stages in the development of the embryo. FIGS. 28–33 × 1500, FIG. 34, × 1200, FIG. 35, × 750, FIG. 36.—Longitudinal section of mature seed. × 355.

The chalazal megaspore is always the functional one, and develops into the mature 8-nucleate embryo-sac, while the three micropylar megaspores degenerate. The development of the 8-nucleate embryo-sac is quite normal and Figs. 18 to 21 show the various stages leading upto the formation of the 8-nucleate embryo-sac. Fig. 20 shows two groups of four nuclei at each end of the embryo-sac. Often the polar nucleus from the chalazal end reaches the centre of the embryo-sac earlier than the polar nucleus from the micropylar end (Fig. 21). The polar nuclei are distinctly larger than the other nuclei of the embryo-sac. In the mature embryo-sac, the micropylar part is enlarged and contains the egg-apparatus (Fig. 22). The tapetum surrounds only the non-dilated chalazal portion of the embryo-sac (Fig. 22).

(*c*) *Fertilization.*—Fig. 23 shows the male nucleus about to fertilize the egg. The male nucleus would appear to be spherical. The remains of the pollen tube as well as the degenerated synergids can be seen. It is said that vermiform and spiral-shaped male cells are by far the commonest in Angiosperms. Spherical nuclei, have, however, been occasionally reported. Weinstein (1926) in *Phaseolus vulgaris*, Madge (1929) in *Viola odorata*, Newman (1934) in *Acacia Baileyana* and Raghavan (1937) in *Cleome Chelidonii* are some of the records we have of spherical male nuclei. The male nuclei as well as the egg nuclei appear to be in a resting condition at the time of contact. Such a condition appears to be common not only in Angiospermous families, but also in the Coniferales (Guilliermond, 1933) and some Cycadales (Lawson, 1926). This may not have any phylogenetic significance, since this phenomenon is found in such widely separated families as Oenotheraceæ (Ishikawa, 1918), Hydrocharitaceæ (Wylie, 1923), Orchidaceæ (Pace, 1907) and Capparidaceæ (Raghavan, 1937). The antipodals are ephemeral.

(*d*) *Endosperm.*—The first division of the fusion endosperm nucleus is followed by the formation of a transverse wall, which divides the embryo-sac into two more or less equal chambers, a chalazal one and a micropylar one (Fig. 24). The micropylar chamber now divides followed by a longitudinal wall. In the meanwhile, the nucleus of the chalazal chamber has divided longitudinally to form two cells. These become the two chalazal haustoria and are the first to be differentiated (Fig. 25). The two micropylar endosperm cells undergo a second longitudinal division, the plane of this division being at right angles to the first longitudinal division. As a result, the micropylar chamber consists of two tiers of two cells each. These four cells divide transversely, thereby separating a micropylar tier of four cells from a similar middle tier (Fig. 26). The micropylar tier of four cells form the four micropylar haustoria, which soon fuse into a single tetranucleate haustorial body (Fig. 27). The micropylar haustorium is not very active

and most of the integumentary cells in this region are left undigested. The two uni-nucleate chalazal haustorial cells (Fig. 27) are more aggressive than the micropylar haustorium.

(*e*) *Embryo.*—The development of the embryo commences only after a considerable amount of endosperm tissue has been formed. The first sign of the development of the embryo is the commencement of a marked elongation of the oospore (Fig. 28). After the oospore has elongated considerably, the first division of the oospore takes place, which is followed by the formation of a transverse wall (Fig. 29). The apical or the lower cell forms the embryo proper, while the basal cells form the suspensor. Fig. 31 shows the four-celled linear proembryo. Such a linear arrangement of the four-cells of the proembryo is characteristic of the Scrophulariaceæ. A longitudinal wall formed in the lowermost cell of the four-celled proembryo gives rise to the quadrant stage (Fig. 32). A transverse wall across the quadrants results in the octant stage (Fig. 33). Figs. 34 and 35 show later stages, the differentiation of the primary meristems, and the lobing of the cotyledons. The two cotyledonary lobes grow with the plumule between them. On the opposite end the radicle is differentiated. The vascular supply of the radicle and the hypocotyl become gradually continuous with those of the cotyledons (Fig. 36). Fig. 36 shows the mature seed in longitudinal section. There is no endosperm, the entire seed being occupied by the two cotyledons. The testa is composed of two layers of cells. During the growth of the seed, the tapetum is disorganised and the remaining two layers would appear to persist and make up the testa. The inner layer is composed of large thick-walled cells, while the outer layer is composed of thin walled cells tangentially elongated.

IV. Discussion

Haustoria, chalazal and micropylar, appear to be a constant feature of the Scrophulariaceæ. The form and the extent to which they are developed may vary considerably. In all the genera investigated so far, haustoria have been recorded. Only in *Angelonia* the absence of it was reported (Srinivasan, 1940). Schertz (1919) in the course of his work on *Scrophularia marylandica* observes incidentally that in the " Rhinanthaceæ and other members of the Scrophulariaceæ, micropylar and chalazal haustoria appear to be quite constant characteristics. In *Melampyrnum memorosa*, they are very arborescent, while in some other species only rudimentary haustoria occur. In *Scoparia* no haustoria are noticeable ". We have not been able to investigate *Scoparia* yet. But the suggestion made in the previous paper seems to be justified from the observations made in this genus also. It was suggested that the absence of haustoria in *Angelonia*—the only genus where haustoria was found

wanting—was correlated to the persistence of the synergids. In other words to some extent the synergids took the place of the micropylar haustorium. They increased in size and form after fertilization instead of shrivelling and disappearing as they usually do. In forms where haustoria, micropylar and chalazal, are the rule, their nature is now beyond doubt. They are all endospermal in origin. But there was a time when the micropylar haustoria were regarded as transformed synergids. While therefore the true haustorium cannot be anything but endospermal, the idea of synergids functioning as haustoria cannot be considered new. Thus it is quite likely that in such of those few forms, where true endospermal haustoria do not occur, the synergids come forward and take up the role. This can, however, be generalised only by an examination of other forms like *Scoparia* which are said to possess no endospermal haustoria. The present investigation corroborates the previous finding to this extent, namely, that endospermal haustoria being a feature of constant occurrence, there is nothing abnormal in the behaviour of the synergids. Positive corroboration must, however, await further investigation especially of the genus *Scoparia*.

V. Summary

The haploid chromosome number of *Ilysanthes parviflora* has been determined for the first time, to be thirteen. The development of the anther is described. Meiosis of the pollen mother-cells is normal.

The development of the embryo-sac is described. The integumental tapetum surrounds only the non-dilated chalazal portion of the embryo-sac.

There are two uni-nucleate chalazal haustorial cells, which are more aggressive than the four-nucleate micropylar haustorium.

The development of the embryo is normal.

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ON A TRYPANOSOME FOUND IN THE BLOOD OF *UROLONCHA STRIATA* L.

By José Pereira

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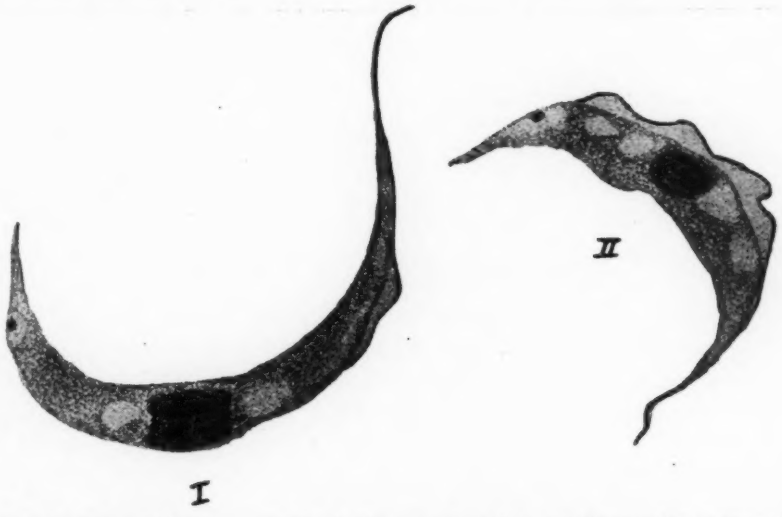
(Communicated by Prof. Col. I. Froilano de Mello)

No parasite has hitherto been recorded in the blood of Uroloncha Striata L. So, the trypanosome here described is a n. sp. which will be named *Trypanosoma Urolonchae*.[1] Only two trypanosomes have been found: monomorphic, with slight individual variations.

Posterior pole expanded in a rostrum-like appendage with a very thin point. The micronucleus roundish, strongly stained in deep violet by May Grunwald-Giemsa, situated at some distance from this point and surrounded by a more or less elliptic vacuole. Nucleus large, stained in rose, situated



nearly in the middle zone of the body, more or less quadrangular, occupying the whole breadth of the parasite and with the chromatin disposed in two or

[1] Beside this trypanosome, *Uroloncha Striata* harbours also an *Hæmoproteus* whose description and evolutice cycle will be dealt with in a further note by Colonel de Mello in collaboration with the present writer and his colleague E. de Figueiredo.

three longitudinal bands. Undulating membrane starting from the micronucleus and with the bordering flagellum well stained in rose in one specimen, scarcely visible only in the anterior third in the second specimen and lacking the rose tinge described above. The free flagellum is very thin and measures circa 1/5 of the legnth of the body. Protoplasm stained in blue, not uniform, but vacuolated, especially near the nucleus.

Measurements in Microns	Specimen I	Specimen II
From the point of the rostrum to the micronucleus	6	4, 5
From the micronucleus to the macronucleus	8, 5	5
Length of the macronucleus	3, 5	2, 5
From the anterior border of the macronucleus to the anterior pole	17, 5	8, 5
Free flagellum	7, 1	5, 1
Total length of the body without free flagellum	35, 5	20, 5
Breadth (without counting the undulating membrane) ..	4, 1	4, 5

THE RANGE OF VARIATION OF NORMAL EYE TENSION AND THE RELATION BETWEEN BLOOD PRESSURE AND EYE TENSION

By T. Prasannasimha Row

(From the Department of Physiology and Biochemistry, University Medical College, Mysore)

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(Communicated by Prof. A. Subba Rau)

Tonometry as an aid in the diagnosis of eye diseases is not of much avail in the absence of information regarding the range of variations of normal eye tension. The measurement of normal eye tension in Europeans (Gjessing, 1905), in Chinese (Paò-Hua, 1932), and in Japanese (Kanda and So, 1933) indicates that the variations are wide. Curiously the highest figure for the normal intra-ocular tension is the same for Chinese, Europeans and Americans; but the lowest is found among Chinese. While the average for Chinese is low, for the Japanese it is the same as that for the Westerners. Corresponding figures are not available for Indians. A reference to literature reveals that extensive investigations have been carried out elsewhere, but not in India, to show the relation between blood pressure and eye tension in some of the clinical conditions where one of them, either blood pressure or eye tension, is altered. The results of clinical research on the relation between osmosis, blood pressure and eye tension show no correlation between blood pressure and eye tension (Weichmann, 1930). Further the observations on the osmotic pressure of the aqueous humour in epidemic dropsy glaucoma (Kirwan and Mukerjee, 1938) and the investigations on the ætiology of glaucoma (Weinstein, 1939) conclusively establish that the variation in blood pressure is not the causative factor in the production of hypertonia in these conditions. But so long as the aqueous humour is considered a dialysate of blood plasma, it is probable that in altered physiological conditions, like pregnancy, the fall in eye tension (Ferrari, 1932) is secondary to the fall in blood pressure (Burwell *et al.*, 1938). On the suggestion of Professor A. Subba Rau the present investigation was undertaken to ascertain the range of variation of eye tension in normal eyes of healthy men and women, and the interrelationship between the blood pressure and the eye tension in men, in normal and in pregnant women living in Mysore.

Persons attending the Ophthalmic Out-Patient Department of the Krishnarajendra Hospital for correction of refractive errors and pregnant

women attending the ante-natal clinic of the Vani Vilas Maternity Hospital (now Cheluvamba Hospital for Women), were the subjects of the present investigation. Care was exercised not to include subjects, who showed signs of suffering either from general diseases like nephritis, hyperpiesia, diabetes, and anæmia or from eye diseases like iritis, cyclitis, ulcer and leucoma of the cornea, which are likely to influence either the blood pressure or the eye tension.

The Schiøtz tonometer with 5·5 gm. weight was used to record the eye tension after the initial smarting of the 1% holocaine solution had passed off. The blood pressure was measured in the recumbent posture by the auscultatory method, using the Tycos aneroid sphygmomanometer.

The results of the investigation are represented in histograms 1 to 6 and Tables I and II. From the values of the statistics g_1 and g_2 and their respective standard deviations, it can be inferred that the tension of the healthy eye is a normally distributed variate in both sexes between 10 and 65 years of age. Of the 210 pairs of eyes examined in healthy men and women, in 41 pairs (emmetropic 38 pairs, myopic 2 pairs, and hypermetropic 1 pair) a difference in the eye tension between the two eyes exceeding 2 mm. of Hg was recorded. The maximum difference observed was 4 mm. of Hg in only one subject, the tension in the left eye (22 mm. of Hg) being the lower.

Figs. 1 and 2 indicate that the average value of the blood pressure is the lowest for the group of subjects below 15 years of age, the highest above 55 years, and between 15 and 55 years shows minor fluctuations with a tendency for it to be higher in the decade between 25 and 35 years than in any other decade in that period. This tendency is pronounced in women,

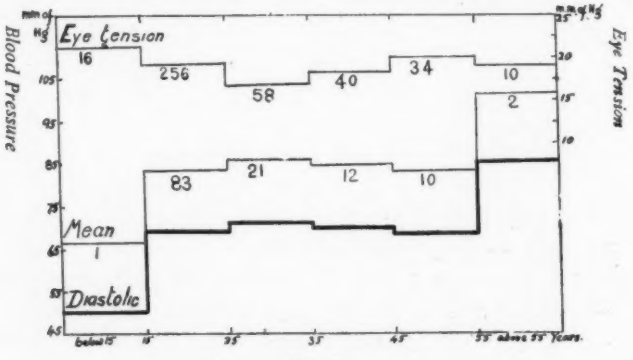

FIG. 1

Blood Pressure and Eye Tension in Men

Numerical figures indicate the total no. of readings in the respective age periods

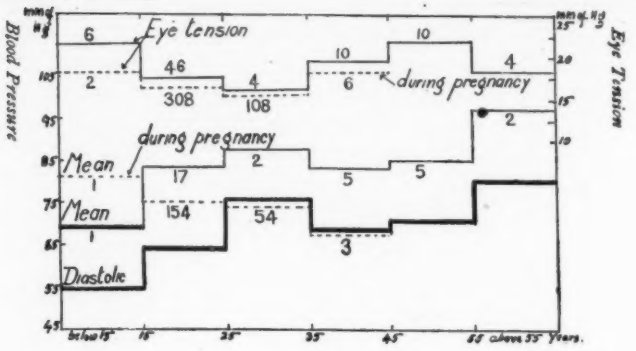

FIG. 2

Blood Pressure and Eye Tension in Women

Numerical figures indicate the total no. of readings in the respective age periods

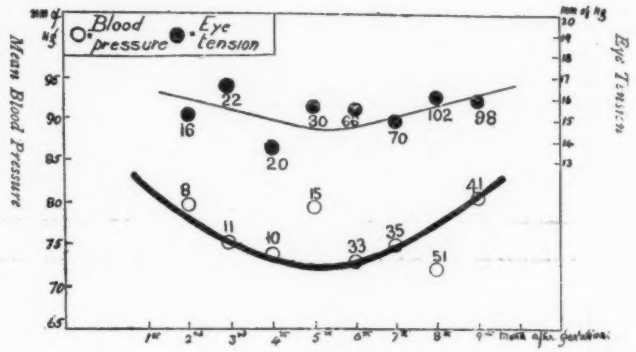

FIG. 3

Blood Pressure and Eye Tension in Pregnancy

Numerical figures indicate the total no. of readings in the respective months of gestation

and particularly well shown in the diastolic pressure. The average value of the eye tension on the other hand is highest before 15 years, and tends to be lower than that value after 55 years in both men and women. In the interval between 15 and 55 years, in both sexes, the fluctuations in the eye tension are opposite to those of the blood pressure (*except in women between* 45 *and* 55 *years, when the eye tension also rises with the blood pressure*) in the corresponding decades of life, but more pronounced in women than in men.

Histogram 1
Normal Healthy Men
$n = 340$; mean = 19·12 mm. of Hg; Std. devn. = 2·90 mm of Hg. $g_1 = 0·3926 \mp 0·2202$; $g_2 = 0·3290 \mp 0·2712$; $Q_1 = 16·04$ & $Q_1 = 20·46$ mm. of Hg.

Histogram 2
Normal Healthy Women
$n = 80$; mean = 18·65 mm. of Hg; Std. devn. = 2·70 mm. of Hg. $g_1 = -0·4550 \mp 0·2709$; $g_2 = 0·2105 \mp 0·5317$; $Q_1 = 16·23$ & $Q_1 = 20·38$ mm. of Hg.

His togram 3
Pregnant Women during the 8th and 9th months of gestation
n 200; mean = 16·5 mm. of Hg; and Std. devn. = 2·32 mm. of Hg.

Histogram 4
Pregnant Women during the 3rd, 4th, 5th, 6th and 7th months of gestation $n = 204$; mean = 15·48 mm. of Hg; Std. devn. = 2·77 mm. of Hg.

Histogram 5
Pregnant Women in all the months of $n = 416$; mean = 16·3 mm. of Hg; gestation Std. devn. = 2·91 mm. of Hg.

Histogram 6
Curve of Relative Frequencies
$n = 420$.

In women, during pregnancy the frequency distribution of eye tension is altered. When pregnant women irrespective of the period of gestation are grouped, the number of subjects whose eye tension is 15 mm. of Hg is smaller than the number of subjects, whose eye tension is either 13 mm. or 17 mm. of Hg (Histogram 5). At the approach of full term, this notch disappears (Histogram 3). Therefore it is only during the early months of pregnancy the reduction in eye tension is very marked (Histogram 4) with a tendency for the mode to be on the left of the mean. During pregnancy the mean of the eye tension is 16·3 mm. of Hg with a standard deviation of 2·91 mm. of Hg, as against 18·65 mm. of Hg ± 2·70 mm. of Hg in normal women. Fig. 2 shows that the eye tension during pregnancy is lower than in normal women in all the corresponding age periods. The blood pressure is also lower than in normal women in the corresponding age periods, except in the case of those pregnant women, who were below 15 years of age. No definite conclusions can be drawn from the above, because of the paucity of numbers in some age periods. The above statements are recorded as indicative of the probable relationship between the variations of eye tension and blood pressure.

TABLE I

Analysis of eye tension with respect to the incidence of refractive errors

Particulars	Sex	No. of eyes	Average eye tension in mm. of Hg	REMARKS
Hypermetropia ..	Male	10	17·6	Of the 207 pairs of eyes examined 5 pairs were anisometropic. One eye in each of the 5 pairs was emmetropic and with respect to the other eye one was hypermetropic in one pair and myopic in four pairs. In two myopic eyes of the 4 pairs the tension differed from the emmetropic eye (higher in one and lower in the other by 2 mm. or more of Hg). In the case of 33 men (1 hypermetrope, 30 emmetropes, and 2 myopes) and 8 women (all emmetropes) the tension in one eye exceeded that in the other by 2 mm. or more of Hg.
	Female	4	20·3	
Emmetropia ..	Male	280	19·3	
	Female	65	17·4	
Myopia ..	Male	44	20·2	
	Female	11	19·7	

Table II gives the coefficients of correlation between the eye tension and the blood pressure in the several classes. The coefficient is positive in all classes, *except in the case of pregnant women nearing term.* The mean blood pressure (diastolic + 1/3 pulse pressure) has a higher correlation, probably because the intra-capillary pressure varies with the mean pressure (Wiggers,

TABLE II

Significance of the variations in the coefficients of correlation between eye tension and blood pressure in the several classes

Class of subjects	No. in each class (n)	Coefficient of correlation (r) between eye tension and				$Z = \frac{1}{2}\log_e \frac{1+r}{1-}$ for eye tension and		Between eye tension, mean blood pressure and diastolic blood pressure		$\sigma_{z_1 \sim z_2} - (Z_1 \sim Z_2)$ for eye tension and	
		Mean blood pressure		Diastolic blood pressure		Mean blood pressure	Diastolic blood pressure	Variance of $Z = \frac{1}{n-3}$	* $\sigma_{z_1 \sim z_2} = \sqrt{\frac{1}{n_1-3} + \frac{1}{n_2-3}}$	Mean blood pressure	Diastolic blood pressure
		Adjusted for grouping	Not adjusted	Adjusted for grouping	Not adjusted						
1. Normal men ..	125	0·09735	0·09389	0·04673	0·04493	0·09409	0·04493	0·008185	0·2010 for 1 & 2	0·39136	0·39798
2. Normal women ..	34	0·08530	0·08325	0·04201	0·04091	0·08345	0·04091	0·03225	0·1950 for 2 & 3	0·37083	0·36725
3. Pregnant women	208	0·06778	0·06418	0·07633	0·07356	0·06428	0·07376	0·004886	0·1142 for 3 & 1	0·16412	0·19717
4. Pregnant women up to 7th month	103	0·1629	0·1574	0·1545	0·1517	0·15875	0·15289	0·01000	0·1434 for 4 & 5	0·06321	0·060463
5. Pregnant women after 7th month	98	−0·06747	−0·06474	−0·07659	−0·07335	−0·06484	−0·07345	0·01050			

* n_1 and n_2, Z_1 and Z_2 refer to the first and second in the following groups under the class of subjects: 1 and 2, 2 and 3, 3 and 1, 4 and 5.

1937) with the eye tension than the diastolic pressure in all the classes, except in the case of pregnant women when grouped irrespective of the period of gestation. In the earlier months of pregnancy (up to the 7th month) the blood pressure shows a much closer positive correlation with eye tension than in normal health. These variations in the coefficients of correlation amongst the several classes are not statistically significant, because in no case the difference $Z_1 \sim Z_2$ exceeds twice the standard deviation of the difference (last column, Table II).

The average normal ocular tension in the present investigation is definitely lower than the figures reported by Shoji (quoted by Kanda and So, 1933) for the Japanese (24·21 mm. of Hg), which the author says is equivalent to that of Europeans. Müller's (1931) curve of relative frequencies based on the 2000 figures of eye tension reported by Gjessing (1905) and the Histogram 6 are similar to each other but with the difference that the limits in the present investigation are 10 mm. of Hg and 30 mm. of Hg as against 12 mm. of Hg and 36 mm. of Hg given by Gjessing. Pao-Hua (1932) reports that the highest normal tension is 33 mm. of Hg and the lowest 13 mm. of Hg, and that although the highest normal intra-ocular tension among the Chinese is the same as that of Europeans and Americans, the lowest figure is much lower and the average is 4 mm. of Hg lower than those of European and American authors. The average normal eye tension among the subjects of the present investigation appears to be about 1 mm. of Hg lower than that of the Chinese, and the range of variation is also shifted to the left by 3 mm. of Hg. From the evidences in Table I giving the analysis of the figures for eye tension with respect to the incidence of refractive errors, one can infer that the eye tension is not influenced by the refractive condition of the eye. The low tension of high myopic eyes in most cases is concomitant with the myopic lesions (Caso, 1931). The average figure for the eye tension during pregnancy (16·3 mm. of Hg) in the present investigation is also lower than Farrari's (1932) figure (17·3 mm. of Hg) for pregnant women. These differences are only apparent, because the Schiøtz tonometer measures only the impressibility of the cornea, and according to Shope (1932) any given Schiøtz tonometer reading may be produced by any of a considerable number of intra-ocular pressures, and different Schiøtz tonometers in good repair often give different readings when applied to the same eye. Since the same Schiøtz tonometer was used throughout the present investigation, it may be concluded that during pregnancy, the eye tension as measured by the Schiøtz tonometer registers a definite hypotony. Fig. 3 shows that this hypotony is very pronounced about the middle of pregnancy. The blood pressure also follows a parallel curve, and is lowest about the

same period. The following observations which corroborate the above, are recorded in the present investigation. In 19 pregnant women, the eye tension and blood pressure were recorded on more than two occasions at intervals of 4 weeks. 7 of them were in the middle months (from 3rd to 7th) and 12 were in the last three months of gestation. Amongst the former the eye tension registered a constant level in one and a fall in 6 subjects, whereas the blood pressure registered a constant level in 2 and a fall in 5 subjects. Amongst the latter, the eye tension registered a constant level in 4, a rise in 4, and a fall in 4 (the average rise being 3 mm. of Hg per month, and the fall 1·5 mm. of Hg per month), whereas the blood pressure registered a constant level in 3, a rise in 6 and a fall in 3 subjects.

The hypotony in pregnant women lasts only during pregnancy, for it was observed from 94 tonometric readings during puerperium (subjects being the In-Patients of the Vani Vilas Hospital) that during the first 3 days after delivery the average eye tension was 14·8 mm. of Hg, rising to 14·95 mm. of Hg during the subsequent 4 days and by the end of about 10–14 days registering a further rise up to 15·75 mm. of Hg. The observations could not be carried out further to ascertain the time it would take on an average for the eye tension to recover from the hypotony induced as a result of pregnancy, as the patients were being discharged on the 10th day.

Discussion

The ocular pressure is an extremely changeable one influenced by many causes like emotion, effort, muscular contractions, all the reflexes of organic life and cardiac oscillations (Bailliart, 1931). "The normal intra-ocular pressure may be taken to be that evolved as the optimum at which the eye is rendered optically rigid and at which its circulation and metabolism of its tissues can at the same time proceed without disturbance" (Duke-Elder, 1934). The maintenance and the regulation of the normal intra-ocular pressure is governed by one or more of the following factors: the efficiency of (1) the pressure circulation through the canal of Schlemm (Duke-Elder and Duke-Elder, 1932); (2) the drainage of the aqueous through the substance of the cornea *via* the filtration angle (Ridley, 1930); (3) the secretory activity of the ciliary body (Robertson, 1937); and (4) a neuro-humoral mechanism (Elwyn, 1938). Whatever be the mechanism that regulates and maintains the normal intra-ocular pressure, it is not disputed that (besides other factors like the volume of the contents of the globe, the elasticity of the coats of the eye, the colloid content of plasma, and the permeability of the capillaries) the intra-capillary pressure is one of the chief factors responsible for the origin and variations of the intra-ocular pressure.

By the use of Herzog's skin capillary measurement apparatus, evidences are adduced towards the existence of raised intra-capillary pressure in glaucomatous individuals; in general the pressure in the ciliary vessels is found to vary with the pressure in the central artery of the retina, the diastolic pressure in which artery is half of that in the brachial artery under normal conditions; and possibly an automatic mechanism regulates in other conditions, the central retinal arterial pressure causing it to rise seemingly passively with the rise in eye tension; but the slight differences in the blood pressure findings of glaucomatous and non-glaucomatous subjects are not suggestive of hypertonia in the former (Weinstein, 1939).

While the normal blood pressure in pregnant women undergoes no important variations, the central retinal arterial pressure suffers a reduction which loses any significance, when compared with the fall in the eye tension (Ferrari, 1932) (probably because of the automatic mechanism). In contradiction to the above, the pressure in the central retinal artery is reported to be higher than normal and the intra-ocular pressure normal in the late stages of pregnancy (Baratta, 1937). But the systemic diastolic pressure exhibits a definite fall during pregnancy (Burwell and others, 1938). It is therefore doubtful whether the fall in the systemic blood pressure reflected in the retinal arteries indirectly causes a reduction in the eye tension or whether the reduction in eye tension is independent of the variations in the blood pressure during pregnancy.

The low coefficients of correlation between the blood pressure and eye tension probably account for the findings, that indicate the absence of correlation between the eye tension and the blood pressure (Weichmann, 1930), and the want of a definite interrelationship between the intra-ocular tension and the blood pressure of opium addicts, who are vagotonics (Kanda and So, 1933). But Spadavecchia (1937) concludes that the intra-ocular pressure depends on the condition of the arterial bed and that there is a limit of separation between the general arterial pressure and the intra-ocular pressure, which he calls the " Threshold of intra-ocular pressure " or the endocular threshold, the high physiological level being capable of alteration by the various morbid and premorbid local conditions, especially those associated with the arterial system.

Kirwan and Mukerjee (1938) believe that the increase of intra-ocular pressure during epidemic dropsy is probably due to altered permeability of the capillaries. In nephritis with low blood protein the eye tension is normal and in nephrosis the eye tension is found to remain constant during recovery, when blood proteins are being restored to the original level, but the intravenous administration of hypertonic saline (30%) brings about a prolonged

depression of the intra-ocular pressure (Robertson, 1939). In pernicious anæmia, the decrease in the intra-ocular tension seems to bear a closer relationship to the hæmoglobin and red cell count rather than to colour index and white cell count (Suker, 1934).

From the evidences presented above, it seems very likely that, whereas variations in the blood pressure influence the eye tension to little extent, conditions which bring about an alteration in the composition of the blood, have a greater effect on the eye tension. It is probably because of the variations in the blood composition in the different age periods that the variations in the normal eye tension in the corresponding age groups, are opposite to those of the blood pressure (Figs. 1 and 2), in spite of the positive correlation between the two variates (Table II). The significance of the positive correlation therefore seems to lie in the fact that the blood composition remaining unchanged, the variations in the eye tension follow the variations in the blood pressure. If the partial coefficient of correlation between eye tension and blood pressure be determined, eliminating the influence of the changes in the blood composition, the volume of the contents of the globe and the elasticity of the coats of the eye (all of which are very labile) the correlation obtained would be much closer. Therefore it is to be expected, that in the same individual under identical conditions of blood composition, volume of the contents of the globe and elasticity of the coats of the eye, any change in the blood pressure influences the eye tension in the same direction. Such conditions are obtained only during the changes in the blood pressure resulting from the beats of the pulse and the movements of the respiration. The observations of Duke-Elder and Duke-Elder (1931) that the intra-ocular pressure faithfully follows variations in the blood pressure, due to respiratory excursions and pulse beats, even the dicrotic notch being represented in the variations of the intra-ocular pressure, support such inferences.

The explanation for the hypotony of the eye during pregnancy, is therefore to be sought in the altered composition of the blood. Ferrari (1932) believes that the changes in the blood chemistry, slight acidosis, and hyper-cholestræmia, possibly explain the hypotension during pregnancy. Ferraris (1933) attributes the cause of the lowered intra-ocular pressure in pregnant women to any or all the changes in the blood, like hyper-glycæmia and increase of certain hormones in blood. The tendency for both eye tension and blood pressure to return to original levels in the last months of pregnancy, is in conformity with the observations of Cohen and Thomson (1939), that whereas the blood volume, velocity of blood flow, and the cardiac output are increased up to 9th month and decreased prior to

delivery ; the hæmoglobin, red cell count, hæmatocrite reading and viscocity of blood are decreased up to 9th month and increased prior to delivery.

Other things remaining the same the fall in blood pressure alone can bring about the above changes in the blood and its circulation. The capillary pressure is lowered as a result of the general fall in diastolic pressure, resulting in an alteration in equilibrium between the tissues and the blood, so that less fluid transudes from the capillaries into the tissues. This increase in blood volume results in the dilution of the blood constituents like hæmoglobin and red cell count, thus lowering the viscosity of the blood, and increasing the cardiac output and velocity of blood flow. A return of the blood pressure to original levels in the last months of pregnancy brings about a reversal of all the above changes. Therefore the nature of the changes in the blood and its circulation during pregnancy are probably to an extent secondary to the variations in the blood pressure arising from the altered physiology of pregnancy.

Therefore the higher coefficients of correlation obtained in the present investigation between the eye tension and the blood pressure in the earlier months of pregnancy than in normal health—though statistically not significant—are due probably to the indirect influence of blood pressure on eye tension through the changes in the blood composition.

Whereas in the early months of pregnancy up to 7th month, the basal metabolic rate is increased just in proportion to the mass of the growing fœtus, in the later months (the mass of the fœtus being considerable) the basal metabolic rate is increased probably by the overactivity of the thyroid (Wiggers, 1937). Therefore the endocrine activity during the last months of pregnancy is likely to be different from the rest of the period. Consequently the changes in blood composition are also likely to be different and are very likely to alter the eye tension. The rise in blood pressure in the last months of pregnancy should result in an elevation of the eye tension, indirectly by its influence on the blood composition. The combined effect of the rise in blood pressure and the altered endocrine activity in the last months of pregnancy is either to augment or prevent the proportional rise in eye tension with the rise in blood pressure. The negative coefficient of correlation in the last months of pregnancy indicates that the rise in eye tension is not in proportion to the rise in blood pressure. It can therefore be summed up that the altered endocrine activity during the last months of pregnancy has opposite effects on eye tension and blood pressure, tending to depress the former and elevate the latter.

The observations, that in women between 45 and 55 years (in the first half of which decade there is the incidence of climacteric (Wiggers, 1937) usually associated with endocrine disturbances), the eye tension follows the rise in blood pressure in contrast to that obtained in other age periods and in men; as also the finding of a negative correlation between the two, in the last months of pregnancy seem to lend support to the humoral control of the maintenance and regulation of the normal intra-ocular pressure.

Summary

(*a*) The Schiøtz tonometer and the Tycos aneroid sphygmomanometer are used to record the eye tension and the blood pressure of 465 subjects consisting of 170 healthy men, 40 healthy women, 208 pregnant women and 47 women after parturition. The results of the investigation are analysed to ascertain

(1) the range of variation of normal eye tension in healthy men and women (average for men = 19·12 mm. of Hg, $\sigma = \pm 2{\cdot}90$ mm. of Hg and for women = 18·65 mm. of Hg, $\sigma = \pm 2{\cdot}70$ mm. of Hg the range being 10 mm. to 30 mm. of Hg);

(2) the influence of age on the variations in eye tension and blood pressure, and their interrelationship;

(3) the relation of the hypotony in pregnancy to the reduction in the systemic blood pressure.

(*b*) The following observations and conclusions are drawn regarding (2) and (3):

(*i*) The variations in the eye tension are opposite to those of the blood pressure in all age periods in both sexes, except in women between the ages 45 and 55 years—probably because of the endocrine disturbances accompanying the climacteric.

(*ii*) The eye tension and blood pressure are the lowest about the middle of pregnancy, both varying in a parallel manner from the start to the termination of pregnancy.

(*iii*) The factors influencing the eye tension and blood pressure in the last months of pregnancy have a greater depressing effect on the eye tension than on the blood pressure.

(*c*) The bearing of (*i*), (*ii*) and (*iii*) on the humoral control of the maintenance and regulation of the normal intra-ocular pressure is discussed.

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FACIAL MUSCULATURE OF SEMNOPITHECUS ENTELLUS

By A. Ananthanarayana Ayer, B.A., M.B.B.S.

(From the Department of Anatomy, Andhra Medical College, Vizagapatam)

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Introduction

A recent perusal of Darwin's book, *The expression of the emotions in man and animals* (1890), stimulated the author's interest in the facial musculature of the primates. On looking into the available literature on facial musculature and the extensive bibliography therein cited, it became obvious that though the facial muscles of many Cercopithecidæ were already worked out in detail, the study had been confined to the Cercopithecinæ. No mention was found anywhere to the arrangement of the facial muscles in the Semnopithecinæ, nor any diagram among the hundreds given by Edgeworth (1935). Ruge's classical work has not been available here; but Polok (1908) has stated that Ruge did not study Colobus or Semnopithecus.

Another point also became clear. Though Ruge (cited by Lightoller, 1928) was unwilling to allow, probably on account of the cheek pouches, that the Cercopithecidæ belonged to the anthropoid stem, and hence regarded them as unsuitable for purposes of comparison, Paugger (cited by Lightoller, 1928) does not agree with the attitude of Ruge and Lightoller (1928) definitely says: "There seems to be no valid reason, why the appearance of buccal herniæ should prevent the facial musculature of these primates being regarded as, more or less, accurately representing that possessed by the predecessors of the higher primates". If the facial musculature of these Cercopithecidæ with a buccal pouch should have such comparative value according to Lightoller, it naturally follows that the facial musculature of those Cercopithecidæ without the buccal pouch should have an enhanced comparative value. The Semnopithecus entellus is one such, and also it has a relatively foreshortened muzzle. As the facial musculature of the Semnopithecinæ has not been described before, the present work has been undertaken.

Materials and Methods

Two specimens of female laugur (*Semnopithecus entellus*), young adults, embalmed according to the usual technique followed in the Department of Anatomy, Andhra Medical College, Vizagapatam, have been dissected.

Classification and Terminology

The muscles have been classified on a regional grouping as generally followed in anatomical text-books. As regards nomenclature it was found that the terminology available in human anatomy was not sufficient and names had to be borrowed from comparative anatomy. Instead of creating a new mixture, it was considered convenient to follow a standard already set and the nomenclature used by Lightoller (1928) has been uniformly adopted.

M. Subcutaneous Colli—

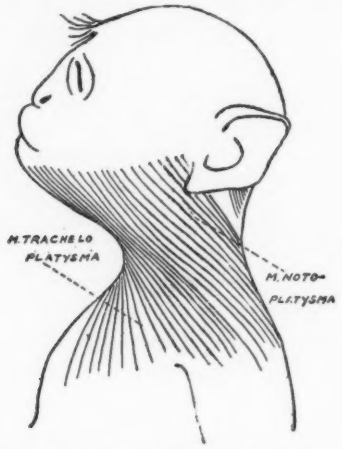
M. Platysma.—(Text-figs. 1, 2, 3, 4). The subcutaneous musculature of the neck is represented only by the platysma. The sphincter colli superficialis and the sphincter colli profundus are both absent. The platysma forms an extensive and very nearly complete investment for the neck all round. There is no apparent demarcation that can be made out between

TEXT-FIG. 1. Dissection of face and neck showing m. subcutaneous colli, anterior view.

the noto-platysma and the trachelo-platysma either by difference in plane or direction of muscle fibres or by intermuscular gap between the muscles. So a conventional demarcation between the two muscles has to be made. "The tip of the acromion process will be regarded as the point where the noto-platysma and the trachelo-platysma meet" (Lightoller, 1928).

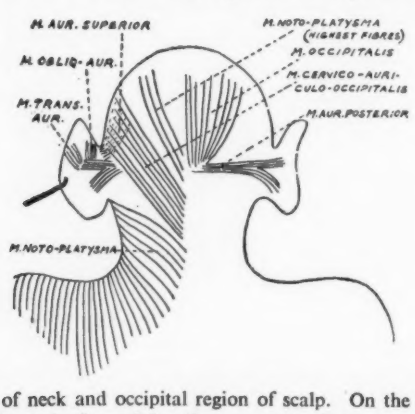
M. Noto-platysma.—(Text-figs. 1, 2, 3, 4). The muscle fibres take origin close to the mid-dorsal line of the neck and the lower part of the line of origin deviates laterally over the supraspinous region and shoulder. Their course and insertion can be conveniently described in three groups: (1) The highest muscle fibres, 3 or 4 discrete muscular fasciculi, from the

upper part of the origin, pass cephalad constituting a superficial continuation of the noto-platysma to the occipital part of the scalp. (2) The muscle fibres



TEXT-FIG. 2. Dissection of the face and neck showing m. subcutaneous colli, side view.

taking origin from the upper half of the mid-dorsal line of the neck pass upwards and laterally to be attached to the auricle and also to join the posterior part of the auricularis superior and the galea. This muscle is the Cervico-auriculo-occipitalis and though on surface view this appears to lie on the same plane as the layer constituted by the highest muscle fibres of the noto-platysma, the Cervico-auriculo-occipitalis in spreading upwards really



TEXT-FIG. 3. Dissection of neck and occipital region of scalp. On the left side the superficial strata of the scalp formed by the m. noto-platysma, and also the intrinsic muscles of the cranial side of the auricle are seen. On the right side the superficial strata have been removed.

forms a substratum to it. (3) Muscle fibres from the lower half of the mid-dorsal line of the neck and from the supraspinous region and the region of the shoulder pass upwards and forwards below the ear over the anterior part of the neck on to the face constituting the supra-angular, mandibular, modiolar and labial parts of the platysma. The supra-angular part extends on the face up to a line from the ear to below the angle of the mouth and partly overlaps the lower part of the zygomaticus. The mandibular part consists of a few fibres attached to the mandible. The modiolar part is attached to the modiolus and also the lower part of the buccinator. The labial part forms the labial tractors for the lower lip.

In addition to the groups of muscle fibres that have been described above as noto-platysma, a deeply separated off-shoot from it is said to have given rise to the auriculo-occipitalis proprius consisting of the auricularis posterior and the occipitalis (proprius). These muscles will be described subsequently.

Trachelo-platysma.—(Text-figs. 1, 2, 4). The muscle fibres take their lower attachment from the region of the shoulder in front of the acromion, from the lateral pectoral region reaching down to the anterior fold of the axilla and from the medial pectoral region as far down as the second intercostal space. There are no muscle fibres from over the body of sternum and manubrium sterni. The muscle fibres proceed upwards and medially and meeting the fibres of the opposite side constitute a strong median decussation extending from the mandible down to a point 2 cm. above jugular notch. Beyond the median decussation, the fibres of one side pass to the opposite side and continue their course interwoven with the fibres of the trachelo- and noto-platysma of the opposite side up to nearly half-way along the side of the mandible. No regularity of layeral arrangement between the decussating fibres of the right and left sides could be made out.

Evolution of the trachelo-platysma.—The trachelo-platysma was regarded by Ruge as an extension of the noto-platysma. But Lightoller (1928) considers it as a separate though sister muscle to the noto-platysma, probably developing from the same anlage. The subprimates possess only a noto-platysma. In primates the trachelo-platysma appears and gradually replaces noto-platysma which finally disappears in man. Lightoller (1928) says: " the key to the position to be occupied by the platysma of any particular primate was obtained from the point of meeting in the face of the noto- and trachelo-platysmæ, which was determined by following a muscle fasciculus from the acromion to the face ". In Semnopithecus entellus this line reaches the face close to the median line on the same side and is similar to that in Baboon and Macacus. Lightoller suggests that the gradual replacement of the noto-platysma by the trachelo-platysma may be connected with

the assumption of the upright position. He further says: " It is curious how closely, apparently this replacement of the noto-platysma by the trachelo-platysma corresponds to the evolutionary grouping of the primate ". The suggestion becomes clarified if it is amplified as follows. The points of attachment of the muscle fibres should be considered, divorced of the conventional meanings that the terms ' origin ' and ' insertion ' connote. The dynamic action of the muscle fibres in relation to the habitual position of the animal and gravity, is the important factor. In subprimates, which are habitually pronograde, the noto-platysma serves as a support for the tissues of the face and neck acting from the dorsum. In the progressively orthograde anthropoid apes, the trachelo-platysma, constitutes a supporting brace for the superficial structures of the ventral part of the neck and especially of the space below the projecting mandibular shelf, and it acts here from its facial and mandibular attachments. Thus the trachelo-platysma becomes emphasised in them, while the noto-platysma gradually disappears having lost its vital function. Among the early primates, *e.g.*, the Cereopithecidæ, where the pronograde and orthograde attitudes are both habitual to the animal, we get a marked development of both noto- and trachelo-platysmæ, with a shifting complexity of action. The progressively lateral spread of the evolving trachelo-platysma displacing the noto-platysma is probably to be accounted for by the progressive widening of the mandibular arcade.

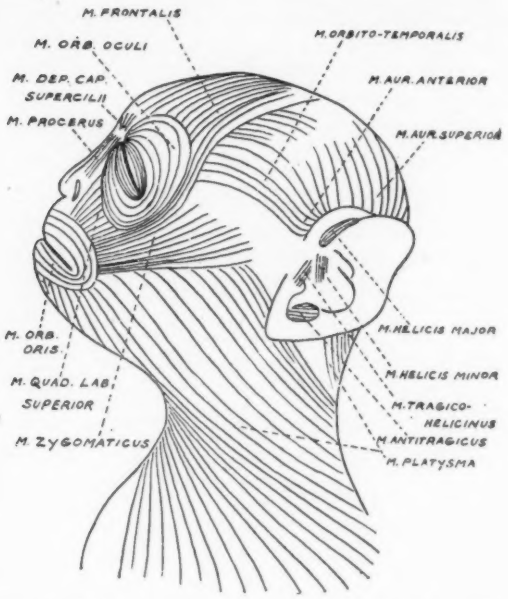
Muscles round the Mouth

M. Quadratus labii inferioris.—This muscle is absent as in Baboon and Macacus.

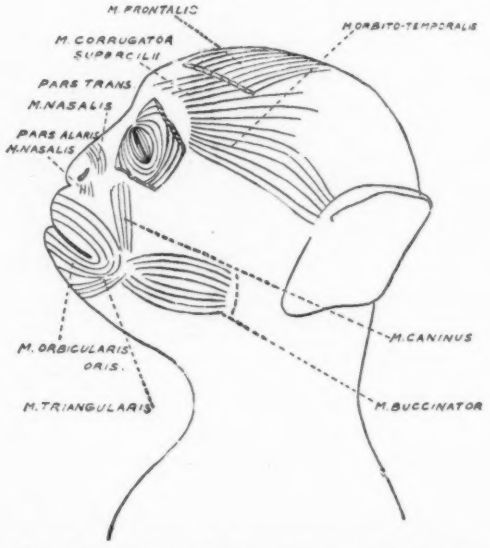
M. Quadratus labii superioris.—(Text-fig. 4). The angular head and the zygomatic head have not become differentiated from the adjacent muscles. The infraorbital head was present passing from the infraorbital margin under cover of the orbicularis oculi into the upper lip.

M. Zygomaticus.—(Text-fig. 4). This muscle takes origin from the external surface of the middle third or more of the zygomatic arch and also from the temporal fascia above. The fibres pass forwards and downwards towards the angle of the mouth to be attached to the modiolus. The upper border of the muscle is adjacent to the lateral border of pars peripheralis orbicularis oculi. The lower part of the zygomaticus covers a part of the parotid gland and is in turn overlapped by the platysma.

M. Caninus.—(Text-fig. 5). It has a linear origin from the maxilla anterior to the jugum for the canine tooth. The fibres pass laterally and caudally to their attachment into the deep part of the modiolus.



TEXT-FIG. 4. Dissection of face and neck showing the superficial musculature of the face and forehead, and also the intrinsic muscles of the lateral aspect of the auricle.



TEXT-FIG. 5. Dissection to expose the deeper muscles of the face and forehead.

M. Triangularis.—(Text-fig. 5). In its complete form when seen in man the triangularis is said to have a deeply situated caput longum and a superficial fan-shaped part subdivided into three rays, *viz.*, caput latum, caput transversum and caput buccale. In Semnopithecus entellus the superficial part is not present. The deep part, the caput longum, is present and is attached to a tubercle on the middle of the outer-aspect of the mandible below the first premolar tooth. It forms a distinct rounded bundle passing obliquely cephaled and laterally into the deep part of the modiolus. Fibres of the caninus and triangularis intimately meet here. Muscle fibres of the labial part of the platysma partly overlap and partly pass deep to the triangularis.

Ruge considered the triangularis as a caudal extension of the fibres of the caninus. Huber (1933) also takes the same view. But Lightoller (1928) considers triangularis as an off-shoot of the trachelo-platysma. The picture of caninus and triangularis in Semnopithecus as seen in Text-fig. 5 is comparable with that seen in the figure of the human embryo given by Futamura reproduced as fig. 378 in Keibal and Mall, *Manual of Embryology* (1910). Futamura regarded the m. triangularis as associated with the m. caninus. It is possible that the caput longum of the m. triangularis originates from the same rudiment as the m. caninus and that the superficial heads are derivatives of the platysma.

M. Risorius.—It is absent.

M. Buccinator.—(Text-fig. 5). The buccinator takes its usual origin from the posterior portion of the alveolar margin of the maxilla and mandible and from the pterygomandibular ligament. In the Macacus, a gap in the continuity of the mandibular attachment about the premolar region is described (Huber, 1933; Lightoller, 1928). But in the Semnopithecus entellus there is no such gap. The fibres of the buccinator proceed forwards to their attachment to the modiolus and some continue into the orbicularis oris.

M. Orbicularis Oris.—(Text-figs. 4, 5). It consists of the usual orbicular fibres getting reinforcement from the incisivi superioris and inferioris and at each modiolus from the buccinator. The orbicular fibres get crosswoven in different planes with the labial tractors.

M. Incisivi Superior and Inferior.—The muscle fibres take origin from the incisive region of the upper and lower jaw close to the mucous membrane of the mouth and the muscle fibres pass laterally uniting with the deeper fibres of the orbicularis oris.

M. Mentalis.—It takes origin from an expanded area on the juga of the lateral incisor and canine teeth of the lower jaw and fibres pass forwards and

upwards through the portio-decussata of the platysma to get attached into the deep aspect of the skin near the median line.

Muscles of the Nose

M. Procerus.—(Text-fig. 4). The muscle is cranially continuous with the frontalis. The muscle fibres pass down on the nose between the orbicularis oculi muscles on either side and overlap inferiorly the pars transversus m. nasalis. The lateral margin of the procerus spreads beyond the nose and gets attached into the upper lip by the side of the quadratus labii superioris.

Pars Transversa M. Nasalis.—(Text-fig. 5). This muscle was absent in the Orangs, Baboon and Macacus dissected by Lightoller (1928). It is, however, present in Semnopithecus entellus in which it forms a transverse muscular band about 3 mm. wide taking origin from the side of the bony piriform aperture medial to the caninus, and passing over the side of the nose to meet the muscle of the opposite side on the dorsum of the nose. The fibres are muscular throughout.

Pars Alaris Proprius M. Nasalis.—(Text-fig. 5). The muscle consists of short fibres passing between the lower part of the piriform aperture and the rudimentary ala of the nose.

Muscles round the Eye

Pars Palpebralis M. Orbicularis Oculi.—(Text-fig. 4). It consists, as usual, of the delicate muscle fibres on the superficial part of the eye-lids.

Pars Orbitalis M. Orbicularis Oculi.—(Text-fig. 4). It consists of the orbicular fibres situated outside the palpebral part and taking attachment from the medial palpebral ligament and the adjacent part of the frontal process of the maxilla. Superiorly the fibres unite with the fibres of the frontalis. At the orbitonasal angle some of the fibres of the orbital part of the orbicularis oculi become differentiated into the depressor capitis (supercilii).

Pars Peripheralis M. Orbicularis Oculi.—(M. Malaris of Henle). (Text-fig. 4.) The medial head of this muscle cannot be differentiated from the adjacent fibres of the procerus and the orbital part of the orbicularis oculi. The lateral head consists of the peripheral muscle fibres of the infero-lateral part of the orbicularis oculi. Craniad, these fibres curve laterally and upwards along the lateral margin of the orbit and then turn upwards and medially. Here they part company from the other orbicular fibres and proceed upwards on the lateral part of the forehead joining the external part of the muscular stratum constituted by the frontalis. Caudad, on the other

hand, some of these fibres pass into the region of the upper lip between the quadratus labii superioris and Zygomaticus.

M. Depressor Capitis (*Supercilii*).—(Text-fig. 4). At the orbito-nasal angle and above the medial palpebral ligament, some muscle fibres are found passing upwards and medially into the fibro-fatty tissue of the medial part of the eye-brow margin and glabella. These muscle fibres are caudally united with the orbital part of the orbicularis oculi.

M. Corrugator (*Supercilii*).—This muscle is situated deep to the orbicularis oculi and frontalis. It takes origin from the glabella and the medial part of the superciliary arch and passes upwards and outwards to become continuous laterally with the orbito-temporalis.

Muscles of the Scalp

M. Epicranius.—(Text-figs. 3, 4). The muscle epicranius consists of the fibro-muscular sheet over the scalp. Anteriorly on the frontal region it shows two muscular strata, *viz.*, a superficial layer, m. frontalis and a deeper layer made up of m. Corrugator supercilii and m. orbito-temporalis. Over the vertex it consists of a single stratum of aponeurotic galea. Posteriorly on the occipital region it consists of three muscular strata, *viz.*, a superficial layer formed by the occipital extension of the notoplatysma, an intermediate layer, the m. Cervico-auriculo-occipitalis and a deeper layer, the m. auriculo-occipitalis (proprius), which is made up of the auricularis posterior and the occipitalis. The individual muscular components are described hereunder.

M. Frontalis.—(Text-fig. 4). The medial fibres of the frontalis muscles of the two sides unite in the middle line and continue downwards as the procerus. The frontalis terminates below deep to the orbicularis oculi in the region of the eye-brow. Laterally the frontalis receives some muscular fibres from the lateral part of the pars peripheralis of the orbicularis oculi. Towards the vertex frontalis ends in an aponeurosis which unites with the deeper stratum to form the galea aponeurotica.

M. Orbito-temporalis —(Text-figs. 4, 5). The deeper muscular stratum on the frontal region consists of the corrugator supercilii medially and in continuation with it laterally, the m. orbito-temporalis. This muscle takes origin from the lateral part of the superciliary arch and external angular process of the frontal bone. The more external fibres of this muscle as they course upwards and laterally have a progressively increasing side deviation so that the most lateral fibres pass in a gentle arc in an approximately horizontal direction over the temporal fascia to the region of the ear, there to become united with the anterior auricular muscle and the anterior part of

the superior auricular muscle. This deep muscular stratum formed by the combined corrugator supercilii and orbito-temporalis muscles ends in an aponeurosis blending with the galea.

Occipital extension of the M. Noto-platysma.—(Text-fig. 3). The highest fibres of the noto-platysma consist of 3 or 4 discrete muscle fibres taking origin from the side of the upper part of the ligamentum nuchæ and proceeding upwards. These have been described as group (1), in the description of the noto-platysma. The individual muscle fibres are too far apart to form a muscular layer. But with the intervening fascia they constitute the superficial stratum of the occipital region of the scalp.

M. Cervico-auriculo-occipitalis.—(Text-fig. 3). It constitutes the intermediate stratum in the occipital region of the scalp. It consists of muscle fibres of the noto-platysma taking origin along the upper part of the ligamentum nuchæ and these fibres were indicated as group (2), in the description of the noto-platysma. They form a muscular ribbon which passes upwards and laterally. The outer fibres get attached to the upper part of the root of the auricle and the other fibres blend over the posterior part of the m. auricularis superior.

M. Auriculo-occipitalis Proprius.—(Text-fig. 3). The third or deepest stratum of the occipital region consists of the auricularis posterior and the occipitalis. The auricularis posterior is described with the extrinsic muscles of the ear. *The occipitalis* takes origin from the medial part of the highest muchal line and passes upwards deep to the two other strata. Where the muscle fibres of the occipitalis become aponeurotic the two superficial strata join it to form a composite galea.

The epicranial musculature of the Semnopithecus therefore presents its primitive components better than the Baboon and the Macacus.

Extrinsic Muscles of the Auricula

M. Auricularis Posterior.—(Text-fig. 3). It consists of a well marked muscular bundle taking origin from the highest muchal line close to the origin of the occipitalis and passing to the posterior part of the concha to be inserted in two well defined bands. The upper band is practically continuous with the muscle transversus auriculi.

M. Auricularis Superior and Anterior.—(Text-figs. 3, 4). These muscles together form a common muscular fan in front of and above the auricle. The anterior muscle fibres become blended with the orbito-temporalis. The posterior part of the auricularis superior becomes united with cervico-auriculo-occipitalis.

The Intrinsic Muscles of the Auricle

M. Transversus Auriculæ.—(Text-fig. 3). It appears to be a continuation of the auricularis posterior; it is directed upwards and backwards on the cranial aspect of the pinna.

M. Obliquus Auriculæ.—(Text-fig. 3). It takes origin from under cover of the upper border of the auricularis posterior and passes upwards.

M. Helicis Minor.—(Text-fig. 4). This small muscle lies on the crus helicis as in man.

M. Helicis Major.—(Text-fig. 4). It is situated on the anterior margin of the helix and is attached to spina helicis.

M. Antitragicus.—(Text-fig. 4). It lies on the antitragus.

M. Trago-helicinus.—(Text-fig. 4). It represents the tragicus but has extended its attachment to bridge over the gap between helix and tragus.

Summary and Conclusion

Two specimens of Semnopithecus entellus have been dissected. The subcutaneous colli consists of well developed noto- and trachelo-platysmæ and forms a nearly complete investment for the neck. The only gaps in this muscular investment are a small triangular interval above the manubrium for a distance of 2 cm. and another smaller triangular gap behind the ear between the fibres of the noto-platysma that go ventral and dorsal to it. The highest fibres of the noto-platysma spread over the occipital region of the scalp in primitive fashion. The quadratus labii inferioris is absent. The quadratus labii superioris lacks differentiated angular and zygomatic heads. Zygomaticus, caninus, the caput longum of the triangularis, the incisivi, and mentalis are present. The risorius is absent. The orbicularis oris and buccinator present the usual features. The procerus overflows from the nose on to the upper lip and is adjacent to orbicularis oculi and infra-orbital head of the quadratus labii superioris. Pars transversa and pars alaris m. nasalis are seen. The depressor supercilii is intimately connected to the medial part of pars orbitalis m. orbicularis oculi. The peripheral part of orbicularis oculi associates itself with the frontalis above and the tractors of the upper lip below. The epicranius shows two muscular strata in front and three muscular strata posteriorly uniting to form the galea over the vertex. The two anterior layers are the frontalis superficially and the combined corrugator supercilii plus orbito-temporalis deeply. Over the occipital region the three layers of the epicranius are, firstly, the highest fibres of the noto-platysma, secondly, the muscle cervico-auriculo-occipitalis, and thirdly, the layer composed of the occipitalis and the auricularis posterior. Over the auricle,

the derivation of the transversus auricularis from the m. auricularis posterior is clearly indicated in the continuity of their fibres. Other extrinsic and intrinsic muscles of the ear are also described.

After a study of the facial musculature of the Baboon and the Macacus, Lightoller (1928) finds that the cercopithecidæ are lavishly endowed with facial muscles and that no support was found for the statement that their facial musculature was altered by their possessing buccal herniæ. His conclusion is that: "They probably would be a much better standard for the facial musculature of the primates than the Lemuroidea." The present investigation has shown that this conclusion is applicable, with even more appropriateness, to that subfamily of the cercopithecidæ without the buccal pouch, of which Semnopithecus entellus is the type.

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STUDIES IN THE GENUS *COLLETOTRICHUM*

1. Saltation in *Colletotrichum capsici* (Syd.)

By T. S. Ramakrishnan, M.A.
(*Agricultural Research Institute, Coimbatore*)

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During the course of studies on different species and strains of *Colletotrichum* occurring in Madras it was frequently noticed that in Petri dish and test-tube cultures, many of them produced a number of saltants. These were quite different from the original cultures showing variations in the nature of the growth, sporulation colour and setæ. Saltation has been recorded in some species of *Colletotrichum* by Bayer, Dastur, Edgerton, Stevens, Chaudhuri and others (Chaudhuri, 1924). In the course of the present investigations saltants were often noticed in *C. capsici*, *C. indicum* and other strains isolated from different hosts. A comparative study of the saltants of one strain isolated from wilting safflower (*Carthamus tinctorius*) plants was made with the idea of examining the extent of variations in morphological and pathogenic characters and the results are recorded in this paper.

The first saltant of this was formed on Richards' agar in the form of narrow sectors radiating from the centre of the Petri dish. Subsequently many others developed on other media also. More saltants developed on Richards' and french bean agars than on quaker oats agar. Brown (1926) has observed that *Fusarium* produced saltants freely on Richards' agar and that " a highly concentrated synthetic nutrient predisposes strongly to saltation ". The cultural studies of this fungus show that french bean and quaker oats agars which are usually used for keeping stock cultures cannot be safely used for maintaining cultures of *Colletotrichum* as saltations may occur readily on these media.

The saltants were usually in the form of fan-shaped sectors and formed singly or in numbers in a dish (Pl. I, Figs. 1–4). These started from the centre or near the periphery. In test-tubes also saltants developed in the form of sectors starting from the point of inoculation but for want of space the forms could not develop as well as in a dish. Yet the line of demarcation between the parent and the saltant could be seen.

Four saltants and the parent strain were taken up for comparative study. The parent is referred to as C and its saltants as C_1, C_2, C_3 and C_4. After the first isolation single spore cultures of the saltants were made except in C_2 and from these further subcultures were obtained. C may be described as an ever saltating strain giving rise to a number of saltants on different media. There was great difficulty in keeping this pure. C_1 was the first saltant to appear on a medium of 2½% glucose and 2% agar, two months after the isolation of C. C_2 developed on quaker oats agar as a sterile form without any acervuli and even when subcultured on other media it maintained its sporeless character. C_3 was formed on Richards' agar as a more intensely sporulating form than the parent. C_4 also originated on Richards' agar, as a sterile sector. When transferred to french bean agar however it produced numerous smaller acervuli. C_1, C_2 and C_4 rarely produced further saltations but C_3 gave rise to a number of saltants on Richards' and french bean agars. Some of the later saltants of C resembled those produced originally. Two similar saltants (sporeless forms) were given off by C and C_3.

Macroscopic Characters.—The macroscopic growth characteristics of the parents and the four saltants on some of the media in Petri dish cultures were as follows:

French Bean Agar (9 days old)

C. Aerial growth of loose grey mycelial zones alternating with acervular rings, acervuli fairly numerous, salmon pink in the middle and black towards the margin of the dish.

C_1. Aerial growth scanty, in the centre, mostly submerged, zones faintly seen, acervuli numerous distributed throughout and minute.

C_2. Very little of aerial growth, mostly submerged, matted, white with radiating lines, no acervuli, centre sometimes light brown.

C_3. No aerial growth, zones visible, acervuli very numerous, bigger than in C_1, flesh pink in the centre due to spore formation and blackish along margin.

C_4. Aerial growth whitish, not very profuse, zones visible, acervuli numerous in rings, minute, brown in colour.

Richards' Agar (10 days old)

C. Aerial hyphæ in zones, pale vinaceous drab, later on may extend over whole surface and mask zonation, acervuli in rings at first but later form a compact layer on the surface of the medium mixed with stromatoid

bodies, the central portion covered with hydrangea pink masses of spores and outer black.

C_1. Little of aerial growth, mostly submerged compact growth, faint zones, acervuli minute, numerous, buff pink.

C_2. Submerged, compact growth, sometimes clumps of hyphæ sticking up, zones absent, cinnamon rufus mixed with whitish areas, no acervuli.

C_3. No aerial growth, zones visible, the whole dish a mass of acervuli, colour varies with age, brick red centre, coral pink outside, jasper pink outermost, acervuli bigger in centre and smaller outermost.

C_4. Good mealy aerial growth, zones visible, white and smoke-grey zones, compact growth, no acervuli.

Quaker Oats Agar (11 days old)

C. Little aerial growth in rings, acervuli numerous, pale flesh colour in the centre, and black towards the margin.

C_1. No aerial growth, faint zones, acervuli numerous, minute pale brownish drab nearer centre and light greyish olive nearer margin.

C_2. No aerial mycelium, zones visible, Hay's russet to Kaiser brown, not uniformly coloured, no acervuli.

C_3. No aerial growth, zones faint, acervuli numerous, light ochraceous salmon mixed with black stromatoid bodies.

C_4. Growth very much resembling C_1, acervuli numerous, minute.

Sterilised Carthamus Stem (20 days old)

C. Whitish aerial hyphal growth mixed with numerous black acervuli.

C_1. No aerial growth, numerous minute acervuli, spore masses very light coloured.

C_2. No aerial growth, and no acervuli, a thin mat on the surface.

C_3. No aerial growth, a thick coat of acervuli with pink spore masses.

C_4. No aerial growth, numerous minute acervuli like C_1.

French Bean Fruits (12 days old)

Only three saltants were grown on this medium.

C. Scanty aerial growth, a thick crust of black acervuli.

C_1. Slight aerial growth, numerous minute acervuli.

C_2. Mycelial growth matted, no acervuli.

C_3. No aerial growth, thickly covered with acervuli with shell pink spore masses.

Microscopic Characters.—Differences were also observed in certain microscopic characters of these forms.

TABLE I

Microscopic characters of the parent and saltants on different media

Strain	French bean agar	Richards' agar	Quaker oats agar
C	Acervuli and stromatoid bodies black, setæ in numbers, some pointed and others blunt, black violet in colour, spores and appressoria numerous.	Black acervuli and stromatoid bodies numerous, sometimes clustered, setæ pointed and blunt, blackish violet, spores and appressoria numerous.	Black acervuli and stromatoid bodies, setæ numerous, pointed and blunt, blackish violet, spores and appressoria present.
C_1	Acervuli small, brown, setæ few, lighter coloured, some acervuli without setæ, spores in plenty, appressoria found.	Acervuli small, buff pink, setæ few or absent, lighter coloured, spores and appressoria in plenty.	Acervuli small, brown, setæ few, lighter coloured, spores and appressoria in plenty.
C_2	No acervuli, spores or appressoria.	No acervuli, spores or appressoria, hyphæ hyaline, or reddish brown.	No acervuli or spores or appressoria, hyphæ hyaline and reddish brown.
C_3	Acervuli very numerous, smaller in size than C, stromatoid bodies present, setæ fewer than in C, pointed and blunt, dull-violet black, spores numerous, appressoria very few.	Acervuli and stromatoid bodies present in large numbers, setæ lighter than in C, pointed and blunt, dull violet black, spores numerous, appressoria very few.	Acervuli and stromatoid bodies numerous, setæ pointed and blunt, spores in plenty, appressoria very few.
C_4	Acervuli small as in C_1 brown, setæ few or absent, lighter coloured, spores in plenty, appressoria present.	No acervuli or stromatoid bodies, appressoria in plenty, no spores.	Acervuli small, brown, setæ few or absent, lighter coloured, spores in plenty, appressoria present.

General Morphology.—The general morphological characters of the parent strain and the saltants exhibited some interesting features. The hyphæ were of the usual type being hyaline or brown in colour with a number of oil globules in the cells; in C_2 the contents in some hyphæ were reddish. Irregular thin walled swellings were sometimes found.

Appressoria were produced in large numbers in C, C_1 and C_4. In C_3 they were not so common and were usually absent in C_2. These were either aerial or submerged and exhibited a wide variation in shape and size from

single-celled rounded ones to moniliform or irregularly branched groups. In the earlier isolations of C a black crust formed along the edges at the junction of the agar and the glass. These were found to be made of plate-like masses of appressorial chains. After continuous growth in artificial media these did not develop. When the spores were kept for germination on slides appressoria were formed at the end of the spore or germ tubes.

The acervuli showed various degrees of diminution in size and variation in colour in the saltants as compared with the parent. In some cases a number of them were clustered together. The number of acervuli formed was influenced by the medium. C formed more acervuli on Richards' agar than on french bean and very few on Brown's agar. C_4 did not form any acervuli on Richards' agar while many developed on french bean and quaker oats agars.

Stromatoid bodies are formed in large numbers especially in C and C_3 and are black in colour. In C_1 and C_4 they are usually not so numerous and are lighter-coloured. In general they resemble the stromatic base of the acervuli but lack conidiophores and conidia though sometimes they bear setæ. Both the acervuli and the stromatoid bodies appear to be homologous in origin.

The greatest variation was however found in the case of setæ. The setæ in C and C_3 may be divided into two classes. One type is much elongated, of a blackish violet colour thicker at the base and tapering towards the apex, septate and thick walled. The other type is shorter less deeply coloured and the apex is much lighter in colour and rounded (Pl. I, Fig. 8). Each acervulus contains a number of setæ of both kinds the relative proportion of the two varying in different acervuli. In C_1 and C_4 the setæ are much fewer, smaller and lighter in colour, and only one or two setæ may be present in each acervulus or they may sometimes be absent (Pl. I, Fig. 7). The walls are very thin and the setæ are easily bent or folded in the middle. The size of the setæ appears to be a widely varying factor and much dependence cannot be placed on this as a distinguishing feature between species. In the same strain differences are found in the size and number of setæ per acervulus. External factors also influence the size greatly. Ikata (1937) working on *Glomerella gossypii* and *Glæospoirium kaki* has observed that setæ formation is affected by environmental conditions and that they cannot be used for taxonomic purposes. The following table gives the measurements of the setæ of three strains taken at different times on different media. The width represents the measurements of the thickest portion at the base. In each case 150 measurements were made.

TABLE II

Measurements of the Setæ on different media

Strain	Medium	Age in days	Mean size in μ	Range in μ
First isolation C	Carthamus tinctorius		168·28 × 6·1	93·60 – 280·8 × 4·68 – 8·58
	Brown's agar .	20	149·2 × 5·42	85·8 – 257·4 × 3·12 – 7·80
	French bean agar ..	,,	193·32 × 5·76	112·32 – 305·76 × 3·12 – 8·58
	Richards' agar ..	,,	187·76 × 4·88	85·8 – 343·2 × 3·12 – 6·24
	Quaker oats ,, ..	,,	192·93 × 6·33	109·2 – 276·12 × 4·68 – 7·8
	French bean ,, .	22	223·1 × 6·5	137·28 – 318·24 × 5·46 – 7·8
	Richards' ,,	28	212 × 5·49	124·8 – 330·72 × 3·9 – 7·02
	,, ¼ Normal	11	226·28 × 5·68	171·6 – 304·2 × 4·68 – 8·58
	,, ½ Normal	12	182·04 × 6·01	124·8 – 237·12 × 4·68 – 7·8
	,, Normal	13	168·34 × 5·96	117·0 – 241·8 × 4·68 – 7·8
C_1	Quaker oats agar	20	106·84 × 3·60	81·12 – 163·8 × 3·12 – 4·68
	French bean ,, ..	26	85·64 × 3·43	43·68 – 135·72 × 3·12 – 5·46
	Richards' ,, ..	27	137·22 × 3·92	88·92 – 174·72 × 3·12 – 4·68
	,, ¼ Normal	14	117·37 × 3·70	78·0 – 143·52 × 3·12 – 4·68
	,, ½ Normal	14	138·61 × 3·84	93·6 – 171·6 × 3·12 – 4·68
	,, Normal	14	133·01 × 3·95	99·84 – 201·24 × 3·12 – 4·68
	,, ,,	18	158·25 × 3·99	106·08 – 199·68 × 3·12 – 4·68
	,, ,,	21	147·48 × 4·23	93·6 – 179·36 × 3·9 – 5·46
C_3	Richards' agar 1st isolation ..	13	115·15 × 5·72	74·88 – 204·36 × 4·68 – 7·8
	Richards' agar ..	18	149·03 × 4·99	98·28 – 202·8 × 3·12 – 7·02
	Brown's ,,	18	159·24 × 4·65	112·32 – 280·8 × 3·12 – 7·8
	Richards' ,, ..	20	137·92 × 6·45	101·4 – 205·92 × 4·68 – 7·8
	Brown's ,, ..	21	155·38 × 4·89	93·6 – 218·40 × 3·9 – 6·24
	Inoculated plant ..	12	144·99 × 6·35	90·43 – 232·4 × 4·68 – 8·58

Another interesting feature exhibited by some of the setæ of C was the formation from the terminal portion of a cluster of hyaline or slightly coloured branches resembling the conidiophores. The cluster was either sparse made up of a few branches or dense with numerous branches (Pl. I, Fig. 9). Such setæ were lighter in colour. Spores were developed from the tips of these. Archer (1926) states that setæ and conidiophores are homologous in origin. Here it is found that besides being of the same origin they sometimes perform similar functions also.

Spore formation begins from the third day onwards. The conidia are hyaline and curved tapering towards both ends, one end being more pointed than the other. When young they have granular contents but older spores are much vacuolated (Pl. I, Fig. 6). They are usually formed on the acervuli but in C_1 they also develop in clusters at the tips of hyphal branches. The colour of the spore masses varies in different strains and on different media. The saltants exhibit differences in the intensity of sporulation. C_3 is more intensely sporulating than C while C_2 did not produce spores on any media. C_4 produced spores on some media but not on Richards'. Spore lengths differ in the saltants and the medium also influences the size of the spore. In the following table are given the mean length and width of conidia (average of 150 measurements).

In C, C_1, C_3 and C_4 the spores obtained from the host plant or host tissue are distinctly shorter than those from Richards' or french bean agars. The influence of the medium on the spore size in *Colletotrichum* has been noted by Burger (1921).

The parent strain as well as several other saltants exhibited zonated growths. The number of zones corresponds to the number of days' growth—one zone being formed for each day. In C the acervuli first develop in rings alternating with zones of loose aerial hyphæ. At this stage when the dishes are observed from the reverse side the acervular rings look like black rings, alternating with colourless rings. Later on however acervuli are formed in the intervening spaces also and the zonation becomes masked especially from the undersurface. Zones are formed by C_1, C_3 and C_4 also. C_4 shows in the beginning the alternation of acervular and mycelial rings on french bean agar alone. Alternation of light and darkness during day and night is found to influence the formation of zones. Two series of Petri dishes were inoculated with C and C_3 and kept side by side near a glass window, one covered with black paper and the other exposed so that light alone was the varying factor. When examined after six days zones were found in the exposed series and were absent in the covered ones.

TABLE III

Measurements of conidia of C and its saltants on different media

Strain	Medium	Age in days	Range of length in μ	Mean length in μ	Mean width in μ
C	Host plant ..	—	18·72— 31·2	25·32	3·36
	Brown's agar ..	20	20·28— 35·90	27·78	3·36
	French bean agar ..	20	20·28— 32·76	28·92	3·24
	,, ..	22	24·96— 37·44	31·17	3·36
	Richards' agar ..	13	21·84— 37·44	29·97	3·14
	,, ..	28	23·4 — 37·44	30·00	3·14
	Quaker oats agar ..	11	23·4 — 31·2	27·22	3·12
	,, ..	22	18·72— 32·76	26·64	3·36
C_1	Carthamus stem ..	27	18·72— 28·08	23·31	3·12
	Brown's agar ..	15	18·72— 26·52	22·18	3·93
	French bean agar	7	20·28— 29·64	24·69	3·21
	,, ..	26	20·28— 31·2	26·41	3·40
	Richards' agar ..	15	21·84— 31·2	27·21	3·17
	,, ..	27	18·72— 32·76	26·52	3·34
	Quaker oats agar	11	18·72— 28·08	23·82	3·12
	,, ..	22	17·16— 31·20	25·40	3·36
C_3	Carthamus stem ..	26	18·72— 31·2	28·10	3·12
	Brown's agar ..	15	26·52— 35·9	30·73	3·31
	Richards' agar ..	13	23·4 — 37·44	31·28	3·12
	Quaker oats ..	11	23·4 — 32·76	28·05	3·12
C_4	Carthamus stem ..	27	21·84— 28·08	24·54	3·12
	French bean agar ..	17	21·84— 32·76	28·52	3·16
	Quaker oats agar ..	11	21·84— 29·64	25·10	3·12

Pathogenicity.—The parent and three saltants were inoculated on safflower plants and a few other hosts to find out if there was any difference in their infective capacity.

TABLE IV

Results of Inoculation

Host	C	C_1	C_2	C_3	Control
Carthamus tinctorius seedlings					
Unwounded	6/6	6/6	0/6	5/6	0/6
Wounded	6/6	6/6	1/6	6/6	0/6
Cicer arietinum seedlings					
Unwounded	8/8	0/8	0/8	8/8	0/8
Wounded	8/8	0/8	0/8	8/8	0/8
Dolichos lablab seedlings					
Wounded	0/6	0/6	0/6	0/6	0/6
Green fruits of *Capsicum annuum*					
Unwounded	0/6	0/6	0/6	0/6	0/6
Wounded	6/8	4/8	0/6	4/8	0/6
Bolls of *Gossypium herbaceum*					
Wounded	2/6	0/6	0/6	0/6	0/6
Phaseolus vulgaris fruits					
Wounded	0/8	0/8	0/8	0/8	0/8

(The denominator denotes the number inoculated and the numerator the number infected.)

There is marked difference in the pathogenicity of the different forms. On *Carthamus* seedlings, C, C_1 and C_3 are found to be more or less equally parasitic judged from the number of successful infections. But they differ from each other in speed and spread. C and C_3 spread more quickly than C_1 especially in the unwounded plants. On *Cicer* plants C and C_3 caused a rotting of the tissues which turned black. This spread to other portions and finally the whole plant succumbed. C spread quicker than C_3. With C_2 only one plant in the wound-infection series was affected, and even here the infection did not spread further as in C and C_3. On *Carthamus* and *Cicer* plants prominent acervuli developed on the diseased portions in ten days with C and C_3. With C_1 the acervuli were not prominent and no

acervuli developed in the solitary instance of infection with C_2. On *Capsicum* fruits, cotton bolls and *Phaseolus vulgaris* fruits the infection experiments were conducted inside moist chambers the fruits being sterilised beforehand with ·1% mercuric chloride solution and washed with sterilised water. On green *Capsicum* fruits wound-infections alone were successful. There were greater number of infections with C than with C_1 or C_3. With C and C_3 spots developed on the surface and later acervuli formed on these portions and on the seeds. On cotton bolls in the two cases of wound-infection with C there was a slight discolouration of the pericarp and more discolouration of the lint within. Hyphæ were present inside the lint hairs and few acervuli developed. C_2 behaved as a non-pathogenic form.

Comparative studies between C and *Colletotrichum capsici* isolated from diseased *Capsicum* fruits revealed a close similarity between the two fungi in their morphological, cultural and to some extent pathogenic characters. Hence the fungus C must be referred to *C. capsici*. It was also observed that *C. capsici* from *Capsicum* produced a number of saltations on french bean and Richards' agars, some of them resembling those formed by C.

Many species of *Colletotrichum* have been recorded and the number has increased by the amalgamation of the genus *Vermicularia* with *Colletotrichum*. Grove (1938) maintains that the two genera should be kept separate as in the former the setæ are an essential element often produced in large numbers while in the latter setæ are inessential and may be many, few or none. It has been shown above that the frequency of setæ varies enormously in the saltants of *C. capsici* (originally *V. capsici*) sometimes being even absent and hence setæ cannot be of much taxonomic value, in differentiating genera. Furthermore in the creation of species like *C. curcumæ*, *C. zingiberi* and *C. indicum* great reliance has been placed on the ability to infect particular hosts (Sundararaman, 1922, 1926; Dastur, 1934). *C. capsici* has been found to give rise to a number of saltants which showed differences in pathogenicity and spore measurements, showing that the criteria on which species are differentiated in this genus are not constant characters but liable to variation in the same species. It is suggested that some of the species recorded in this genus are only different strains of the same species having become specialised on particular hosts. *C. capsici* may be considered a polymorphic species constantly throwing off new forms. Furthermore it will be more convenient if the species of *Colletotrichum* that exhibit morphological resemblances are included under one and the same species. Butler (1930) is of opinion that " saltants sometimes differ in virulence from parent strains and that there are various ways in which a fungus may change its host

range. Hence it is difficult to admit that biological specialization alone affords a sufficient basis for distinguishing species ".

Many thanks are due to Mr. K. M. Thomas, Government Mycologist, for advice and help in the preparation of this paper.

Summary

A species of *Colletotrichum* isolated from *Carthamus tinctorius* was found to be *C. capsici*. This gave rise to several saltants in cultures. A comparative study of four of the saltants and the parent was made. The saltants showed differences in growth characters, size of acervuli, presence or absence of setæ and sporulation. The setæ were found to be of two kinds and the size was much affected by environment. The medium influenced the size and production of spores. Saltants differed in their pathogenic ability. It is suggested that in the creation of new species of *Colletotrichum* pathogenic capacity alone should not be given much prominence. *C. capsici* constantly gives rise to new races in cultures.

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EXPLANATION OF PLATE FIGURES

FIG. 1.—C producing a saltant on French bean agar.
FIGS. 2 and 3.—C_3 producing saltants on Richards' agar.
FIG. 4.—C producing saltants on Richards' agar.
FIG. 5.—Acervuli from Carthamus plants. × 200.
FIG. 6.—Conidia from culture. × 500.
FIG. 7.—Small acervuli with one or two setæ of C_4. × 200.
FIG. 8.—Portion of an acervulus of C_3 showing pointed and blunt setæ. × 250.
FIG. 9.—Setæ bearing branches at the tip. × 500.
FIG. 10.—Acervuli of C with large numbers of setæ. × 200.
FIG. 11.—A single acervulus showing setæ of C_3. × 250.

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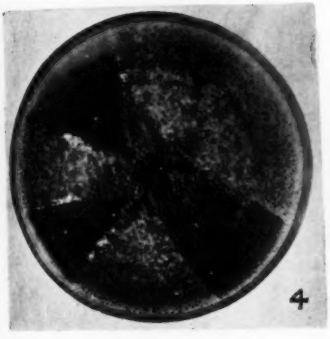
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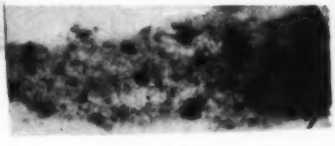

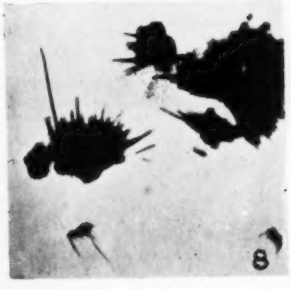
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STUDIES IN THE DISEASES OF *MANGIFERA INDICA* LINN.

Part III. Investigation into the Effect of Sulphur Dioxide Gas on the Mango Fruit

By S. N. Das Gupta, G. S. Verma and S. Sinha

(From the Department of Botany, University of Lucknow)

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I. Introduction

The necrosis (black-tip disease) of the mango fruit is popularly believed to be due to the deleterious effect of fumes arising from brick kilns operating in the vicinity of mango orchards. It was, therefore, thought advisable to ascertain the effect of brick kiln fumes and their constituent gases on the healthy mango fruits in orchards where black-tip disease is unknown. Fumigation experiments, under controlled conditions, were therefore carried out with both coal gas and sulphur dioxide gas which are known to have harmful effects on the vegetation. The present paper deals exclusively with the effect of sulphur dioxide gas on the mango fruits.

II. Material and Method

The investigation was carried out at the Government Horticultural Gardens, Lucknow, in which locality the black-tip disease is not known to occur. Mostly Safeda mangoes were utilised for the experiments as the number of other varieties of fruits available were not sufficient for the purpose. A number of Dasehri fruits were also used.

While still on trees the fruits were enclosed in a gas chamber inside which a known quantity of SO_2 gas was produced by burning carbon disulphide and absolute alcohol.

In the year 1938 a fumigation chamber after the pattern designed by Haywood (1910) for testing effect of sulphur dioxide on young trees was used (Pl. II, Fig. 1). This fumigation chamber measured 3 ft. × 3 ft. × 4 ft. consisting of a wooden frame with glass panes on all sides except the top which was left open. One end of a piece of good rubber sheet was fixed round the open face, the other end of the rubber remaining free to be tied round a twig bearing mango fruits. On one side of the chamber a small window was made which could be closed tightly. But this type of chamber proved inconvenient and had its limitations for our purposes. By using a

big chamber of this type only one concentration of sulphur dioxide could be used in one setting and the manipulation took much time. Using the large fumigation chamber preliminary experiments were made in the mango season of 1938 just to see the effect of sulphur dioxide gas on the fruits. Systematic and more organised work was done in the summer of 1939 when the authors designed their own fumigation chamber which could be worked with more convenience and ease. The latter type of chamber (Pl. II, Fig. 2) consisted of a small rectangular tin cannister measuring 22 cm. × 14 cm. × 14 cm. fitted on one side with glass and on another with a window like arrangement with an air-tight lid. The upper face of the tin cannister was kept open. Round this open face, one side of a good rubber sheet was fixed by means of a tightly screwed iron strip, the other side of the rubber sheet remaining free to be tied round a twig bearing fruits (Pl. II, Fig. 2). The volume of the chamber thus set up measured approximately 9,100 c.c. The arrangement when set up was completely air-tight. These fumigation chambers proved very convenient and useful because of their small size and portability. Moreover on a single day many of these could be utilised using a varied number of concentrations of sulphur dioxide.

Fumigation with Sulphur Dioxide.—For the production of sulphur dioxide gas inside the chamber a mixture of carbon disulphide and absolute alcohol in a given proportion was burnt in a procelain dish or a watch glass kept inside the chamber through the window. The burning liquid could be easily watched from outside through the glass panes. The fumes were allowed to react with the fruits for durations needed; after this period the chambers were removed. The observations were made at definite intervals after the fumigation. In order to confirm results, a large number of twigs bearing fruits were used for testing the effect of a single concentration of sulphur dioxide.

Effect of burning Absolute Alcohol.—Since absolute alcohol was burnt mixed with carbon disulphide in the above experiments, it was thought desirable to find out the effect of burning alcohol on the fruits. The same fumigation chambers were employed and alcohol was burnt in watch glasses. No visible sign of any damage was noticed on the fruits. These experiments along with those in which the fruits were merely enclosed in the fumigation chambers without any thing being burnt inside served as controls.

Study of the Effect of Sulphur Dioxide on the Internal Tissue of Fruits.—Hand and microtomed sections of the affected and healthy fruits were cut to study the effect of the gas on the tissues of the fruits. Epidermal peelings were also utilised to aid the above study.

III. Fumigation with Sulphur Dioxide

During the mango season of 1938 the large fumigation chamber designed after Haywood was used. In order to see the effect of sulphur dioxide on the fruits, carbon disulphide mixed with absolute alcohol was burnt and it was noticed that the effect of the gas was in the production of brick red spots on the skin of the fruits. The spots increase in size as days pass off after the fumigation and in the cases when a large quantity of carbon disulphide was burnt these spots coalesce presenting a more or less uniformly affected surface of the fruit, leaving almost no characteristic green healthy surface. But this condition of the affected fruit in no way resembles the kind of necrosis termed as black-tip disease of mango.

In the following year (1939 mango season) experiments were performed using the improved type of fumigation chamber described above and employing a wide range of sulphur dioxide concentrations. Each set of concentration was tried three times in order to confirm the results. The results are given in Table I.

TABLE I

Table showing Effect of Various Concentrations of Sulphur Dioxide Gas on Safeda Mangoes

Volume of CS_2 burnt	Volume of absolute alcohol burnt	Volume of sulphur dioxide produced	Concentration of SO_2/100	Period of fumigation	Result
c.c.	c.c.	c.c.	c.c.	Min.	
0·05	0·95	42·0	0·46	30	−
0·08	0·92	67·2	0·74	,,	−
0·10	0·90	84·0	0·92	,,	−
0·15	0·85	126·0	1·38	,,	+
0·20	0·80	168·0	1·84	,,	+ +
0·25	0·75	210·0	2·3	,,	+ +
0·30	0·70	252·0	2·76	,,	+ + +
0·10	0·90	84·0	0·92	30	−
0·11	0·89	92·4	1·01	,,	+
0·12	0·88	100·8	1·10	,,	+
0·13	0·87	109·2	1·20	,,	+
0·14	0·86	117·6	1·29	,,	+
0·15	0·85	126·0	1·38	,,	+

It will be seen from the above Table that when mangoes are exposed to 0·92% SO_2 or below this concentration, no spots appear on the surface of the fruits; but at 1·38% SO_2 and upward concentrations the characteristic brick red spots appear.

As it seemed that the effect of sulphur dioxide would depend on the period of exposure, mangoes were subjected to one hour, one and a half hour, eight hours, twenty four hours and for eight continuous days' exposure to varying concentrations of sulphur dioxide. In the last named experiment a fresh supply of SO_2 gas was given every twenty-four hours, after the chamber had been ventilated with fresh air. For obvious reasons the longer the exposure the lesser were the concentrations used. Results of these experiments are given in Table II.

TABLE II

Table showing effect of Period of Exposure for Varying Concentrations of Sulphur Dioxide on Safeda Mangoes

Volume of CS_2 burnt	Volume of absolute alcohol burnt	Volume of sulphur dioxide produced	Concentration of SO_2/100	Period of fumigation	Result
c.c. 0·05	c.c. 0·95	c.c. 42·0	c.c. 0·46	1 Hour	–
0·06	0·94	50·4	0·55	,,	–
0·07	0·93	58·8	0·64	,,	+
0·08	0·92	67·2	0·74	,,	+ +
0·01	0·99	8·4	0·09	1½ Hours	–
0·02	0·98	16·8	0·184	,,	+
0·03	0·97	25·2	0·28	,,	+
0·04	0·96	33·6	0·37	,,	+
0·05	0·95	42·0	0·46	,,	+
0·01	0·99	8·4	0·09	8 Hours	–
0·02	0·98	16·8	0·184	,,	+
0·03	0·97	25·2	0·28	,,	+
0·04	0·96	33·6	0·37	,,	+
0·05	0·95	42·0	0·46	,,	+
0·001	0·099	0·84	0·009	8 Hours	–
0·002	0·098	1·68	0·018	,,	–

TABLE II—(*Contd.*)

Volume of CS_2 burnt	Volume of absolute alcohol burnt	Volume of sulphur dioxide produced	Concentration of SO_2/100	Period of fumigation	Result
0·003	0·097	2·52	0·028	8 Hours	—
0·004	0·096	3·36	0·037	,,	—
0·005	0·095	4·20	0·046	,,	—
0·006	0·094	5·04	0·055	,,	—
0·007	0·093	5·88	0·064	,,	—
0·008	0·092	6·72	0·074	,,	—
0·009	0·091	7·56	0·082	,,	—
0·001	0·099	0·84	0·009	24 Hours	—
0·002	0·098	1·68	0·018	,,	—
0·003	0·097	2·52	0·028	,,	—
0·004	0·096	3·36	0·037	,,	—
0·005	0·095	4·20	0·046	,,	—
0·006	0·094	5·04	0·055	,,	—
0·007	0·093	5·88	0·064	,,	—
0·008	0·092	6·72	0·074	,,	—
0·009	0·091	7·56	0·082	,,	—
0·001	0·099	0·84	0·009	8 Days	—
0·002	0·098	1·68	0·018	,,	—
0·003	0·097	2·52	0·028	,,	—
0·004	0·096	3·36	0·037	,,	—
0·005	0·095	4·20	0·046	,,	—
0·006	0·094	5·04	0·055	,,	—
0·007	0·093	5·88	0·064	,,	—
0·008	0·092	6·72	0·074	,,	—
0·009	0·091	7·56	0·082	,,	—

It will be seen from the above table that on exposure for one hour no effect on the fruits is produced at 0·46% and 0·55% SO_2 but on one and a half hour's exposure, brick-red spots appear on the fruits. Again at 0·09% SO_2 concentration there is no effect when the fumigation lasts for one

and a half hour but after eight hours' fumigation the effect on the fruits is evident. It will be noticed that at certain concentrations, *viz.*, from 0·009% to 0·082% SO_2 the increased period of exposure (varying from 8 hours to eight days) produces no effect. These facts lead to the conclusion that within certain limits of sulphur dioxide concentrations the effect produced on the fruits depends upon the duration of fumigation or *vice versa*, but at certain low concentrations the time factor has no influence.

Since moisture is always present in the atmosphere the possibility of sulphur dioxide gas combining with water and forming sulphurous acid is not remote. The acid thus produced might have something to do with the necrosis of the fruits. In order to find out if the presence of moisture on the surface of the fruit at the time of fumigation would produce any effect on the mango fruits, experiments were carried in which Safeda mangoes were fumigated in the usual way after the fruits were moistened by dipping into water. The results of fumigation of these fruits are given in Table III.

TABLE III

Table showing Effect of Various Concentrations of Sulphur Dioxide Gas on Moistened Safeda Mangoes

Volume of CS_2 burnt	Volume of absolute alcohol burnt	Volume of sulphur dioxide produced	Concentration of SO_2/100	Period of fumigation	Result
c.c. 0·10	c.c. 0·90	c.c. 84·0	c.c. 0·92	Min. 30	–
0·11	0·89	92·4	1·01	,,	+
0·12	0·88	100·8	1·10	,,	+
0·13	0·87	109·2	1·20	,,	+
0·14	0·86	117·6	1·29	,,	+
0·15	0·85	126·0	1·33	,,	+

It will be seen from the above table and Table I that the brick-red spots appear at 1·01% SO_2 concentration whether the fruits are dipped in water or not. The results are the same in the two tables for the corresponding values of sulphur dioxide concentrations. Sulphur dioxide gas, therefore, does not produce any different effect on the fruits in presence of water.

IV. Effect of the Gas on the Fruit Tissue

The external symptom of the gas effect is the production of brown to brick-red colouration around the lenticels on the skin of the fruit (Pl. II,

Fig. 3). This effect is not localised to any one region but extends all over the surface of the mango, wherever lenticels occur. When exposed to lower concentrations of sulphur dioxide gas or when the period of fumigation is less, the brick-red colouration remains restricted to the cells in the immediate neighbourhood of the lenticels having the intervening regions healthy green in colour (Pl. II, Fig. 3). In such cases the effect of fumigation, as revealed by the colouration, does not spread beyond the initial area occupied, even when the mangoes are kept under observation for a long period. In cases of fumigation with higher doses of sulphur dioxide gas (2·3% to 9·3% and oyer) the coloured spots referred to above are of bigger size and as days pass off after fumigation, these affected areas increase in size and coalesce with each other presenting more or less a uniformly affected surface of the fruit, leaving almost no characteristic green healthy portion. But, as pointed out elsewhere, this condition of the affected fruit in no way resembles the kind of necrosis termed as black-tip disease of the mango fruit.

Peelings of the skin of healthy and fumigated fruits were made and compared. These preparations confirmed the results mentioned above and revealed that the gas effect is localised in the beginning around the lenticels, the extent of localisation roughly depending upon the concentration or the period of fumigation of the gas. It was found that the tissue around the lenticels lose the green colour and become light-brown; the extreme periphery of the affected area is conspicuously dark and sharply delimits the latter from the unaffected green portion (Pl. II, Fig. 4). The peelings when examined under the microscope reveal that in the affected area the cells lose chlorophyll, become devoid of starch grains and their walls coloured brown; the cells here and there showing brown deposits. In cases of fumigation with high doses of sulphur dioxide gas all the affected cells show deposits, the latter being more conspicuous and heavier at periphery of the affected areas. Outside the affected region the cells retain chlorophyll and starch grains and remain healthy.

Microtomed and hand sections of healthy and fumigated fruits in various doses of sulphur dioxide gas (1·01%, 1·38%, 1·84%, 2·3%) were cut and studied. It was found that the effect of the gas was most marked just below the lenticels, where the extent of injury is deepest while on the sides it is comparatively less. The affected region thus presents a crescent shape in a section (Pl. III, Figs. 5, 6).

The affected area is more or less sharply marked off from the healthy one by the development of a cambial layer (Pl. III, Figs. 5, 7). The cells between the epidermis and the cambium show injurious effects of the gas;

these become empty, contain few starch grains, their walls coloured brown and in some a brown substance is deposited, the deposition being heavier in the epidermal cells and in the cells just outside the cambium (Pl. III, Figs. 6, 7). In fruits fumigated with a low concentration of sulphur dioxide (*viz.*, 1·01%), the deposits in the affected region are rather inconspicuous and the starch grains continue to occur in the cells (Pl. III, Fig. 5). It is not usual to find, particularly on application of high cencentration of sulphur dioxide gas, groups of the affected cells breaking down leaving empty spaces in that region (Pl. III, Fig. 6).

The formation of the cambium results from the activity of the parenchymatous cells of the mesocarp dividing transversely and adding new cells in which suberisation takes place (Pl. III, Fig. 6) thus forming a layer of cells impervious to gaseous effects and consequently preventing the gas injury from extending further into the deeper tissue of the fruit. The suberisation is quite inconspicuous or even lacking when fruits are fumigated in low concentrations of sulphur dioxide (Pl. III, Fig. 5). The development of the cambium is evidently in response to the injurious effect of sulphur dioxide gas (Butler, 1918; Heald, 1937). It is a device of the tissue to protect itself from the deleterious effect of the penetrating gas. In the case of lower concentrations, when the diffusion of sulphur dioxide gas in solution with the sap or water present in the cell or the cell wall is slow, the cambium layer is formed superficially. When higher concentrations of the gas are applied, the rate of diffusion is faster and before the activity of cambium formation starts the gas has penetrated far inside the tissue and the formation of the cambium takes place in deeper layers of the mesocarp. But whatever be the concentration of the gas the impervious layer of cells are always formed in a semicircle (Pl. III, Figs. 5, 6) around the lenticel, indicating that the gas effect is the most just below the lenticel.

The cells of the mesocarp below the suberised layer remain healthy and unaffected.

V. Discussion

Ranjan and Jha (1940) have recently shown that as a result of the effect of a mixture of sulphur dioxide and air (1 : 1000) the mango fruit becomes pulpy, the green colour gets bleached to some extent and the epicarp becomes loose from the mesocarp, the latter becoming pulpy and brownish. Loose nature of the epidermis was also seen in the control experiments. When mangoes were treated with 0·1% sulphur dioxide for ten days, no blackening was observed but the skin turned whitish through bleaching.

From the investigation carried out here on the effect of sulphur dioxide gas on the mango fruit, it has been seen that the injury produced is first exhibited as small brick-red spots round the lenticels on the skin of the fruit, and of the concentrations of sulphur dioxide, the minimum that produces the effect in 30 minutes' fumigation for one hour is 1·01% SO_2 (Table I). In higher concentrations of sulphur dioxide fumigation the coloured spots referred to above are of bigger size and as days pass off after fumigation, these affected areas increase in size and coalesce with each other presenting more or less a uniformly affected surface leaving almost no characteristic green healthy portion. The effect of sulphur dioxide on mango fruit as observed by the authors thus differs entirely from the observation of Ranjan and Jha (1940). The difference must be due to the difference in the experimental conditions. It is to be noted that the experiments by Ranjan and Jha awere carried out with gathered mangoes while the authors have experimented with fruits on trees.

The first appearance of injury round the lenticels indicates that sulphur dioxide penetrates through the lenticels of the fruits. The effect of sulphur dioxide on the mango fruit will therefore largely depend upon the number, distribution and character of the lenticels. At present no data is available relating to the lenticels. The investigation on this point is being carried out by one of the authors. It should be realised that at that developmental stage of the fruit when the lenticels are present or when the lenticels found in the fruit are totally blocked for any penetration of the gas, the result will be greatly altered both with regard to the nature of the injury it causes and the relation between the minimum concentration of the gas and the period of fumigation required to produce the effect. The results presented here should therefore be true for a particular stage of maturity of the fruit. In a later communication, when the origin and development of the lenticels have been studied, it will be possible to find out the effect of sulphur dioxide gas at various stages of the development of the mango fruit and correlate with lenticel condition.

In the tissue the symptoms of injury consists in the deposition of light-brown substance in the cells and the colouration of the walls in the tissue below the lenticels. The affected region is always in the form of a semicircle in section, the deepest area lying just below the lenticel. The affected portion is sharply marked off from the healthy region by a cambium developed in response to the injurious effects of the gas. The formation of cambium in response to deleterious gases like sulphur dioxide and the invasion of parasitic fungi are well known (Butler, 1918; Heald, 1937). The region of

the tissue in which cambium layer is formed depends upon the concentration and probably the rate of diffusion of sulphur dioxide gas through the tissue. Smaller amount of sulphur dioxide diffusing along through the tissue will give the reacting cells enough time to produce the cambium in upper layers, whereas in the higher concentrations, the gas diffusing more rapidly, will penetrate deep below before the cambium layer is formed. The cambium cuts off one or more layers of cells in which suberisation takes place, the number of layers of these cells depending upon the concentration. The suberised cells prevent penetration of the gas further inside the fruit.

It will be noted that in our experiments the minimum concentration of sulphur dioxide required to produce the symptoms of injury under the experimental conditions employed is 1·01% a value comparatively high to those obtained by Haywood (1910) and others. All the work known relates to the effect of sulphur dioxide on leaf. Haywood (1910) fumigated pine needles and leaves of cowpeas in the various concentrations of sulphur dioxide, giving various periods of fumigation and found positive results for 1·0, 0·1 and 0·01 percentages of sulphur dioxide gas for one hour repeating the fumigation six times, nine times and fifty times respectively. The result of the present investigation shows that the fruits were affected at 1·01% when fumigated for thirty minutes and once only, and 0·09% when fumigated for eight hours; but no effect was perceived in concentrations lower than 0·09% SO_2 even when the duration of fumigation was prolonged for as many as eight days. At certain critical concentration the effect is dependent on the period of fumigation. For example, 0·74% SO_2 for half an hour does not produce any effect, when the same concentration was employed for one hour the effect is evident. Similarly 0·46% SO_2 for one hour has no effect but the same concentration when employed for one and a half hour produces positive results. Below this critical concentration, the time of exposure seems to have no effect although the period of fumigation was sufficiently long. The fruit then appears to be more tolerant to very low concentrations of sulphur dioxide in comparison with leaves of pine and cowpeas. The results obtained with the leaves are not strictly comparable to those obtained with the fruits. Since it is known that the effect of sulphur dioxide on leaves is determined not only by the presence of stomata but also those factors which affect the opening of the same, such as light, temperature, humidity, etc. In the case of mango fruit the conditions are much different, in which the lenticels wherever they occur are not affected by the factors enumerated above. The authors are now investigating the minimum concentration of sulphur dioxide gas which will affect the leaves of *Mangifera indica* Linn. for

various periods of fumigation. The investigation will form a subject of later communication.

The deposits which are found in the epidermal and mesocarp cells of the mango fruit with sulphur dioxide gas, however, are externally comparable with those of the first etiolation stages of black-tip disease. It is difficult however to draw any definite conclusions from this superficial resemblance.

Summary

1. The black-tip disease of mango fruits is believed to be due to the fumes emanating from brick kilns situated near orchards. This paper deals with the effect of sulphur dioxide gas (one of the components of brick kiln fumes) on mango fruits.

2. The fruits while still on trees, were fumigated inside especially designed chambers with known concentrations of sulphur dioxide gas pro duced by burning a given amount of carbon disulphide mixed with absolute alcohol for varying periods ranging from 30 minutes to eight days. The effect of the gas on the fruits were noted and studied by making peelings of the skin and cutting sections of the tissue.

3. The first effect is the production of small brick-red coloured areas round the lenticels all over the skin of the fruit. In the affected region the epidermal cells lose chlorophyll, the starch grains become less in the mesocarpic cells, the cell walls are coloured brown and light brown deposits take place in the cell cavities. The effect of the gas on the tissue is the greatest just below the lenticel and comparatively less on the sides, thus in a section the affected region appears in a semicircle. The affected area is marked off from the healthy tissue by the development of a cambium which produces new cells in which suberisation takes place. This suberised layer is evidently developed in response to the injurious effect of the gas and protects the deeper tissue of the fruit from gas injury. At higher concentrations the formation of the cambium takes place at deeper layers of the tissue. Extent of injury appears to be proportional to the concentration and period of fumigation but after a certain minimum value the rule does not hold good.

Acknowledgment

The authors wish to express their thanks to the Director of Agriculture, U.P., for allowing the use of the mango orchard in the Government Horticulture Gardens, Lucknow, where the fumigation experiment was carried out.

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EXPLANATION OF FIGURES

Plate II

Fig. 1. Large fumigation chamber designed after Haywood, enclosing mango twigs bearing fruits.

Fig. 2. Small fumigation chamber designed by the authors, enclosing a mango twig bearing fruits.

Fig. 3. An entire mango fruit showing the first visible effect of sulphur dioxide injury; H, the green healthy colour, S, the affected areas round lenticels.

Fig. 4. A diagrammatic representation of the affected area (S) on a large scale. L, lenticel. A, light brown area on the skin; B, a dark brown border line delimiting the affected area (S) from the unaffected area G_1 and G_2; G_1, light green healthy skin, G_2 dark green healthy skin.

FIG. 1

FIG. 2

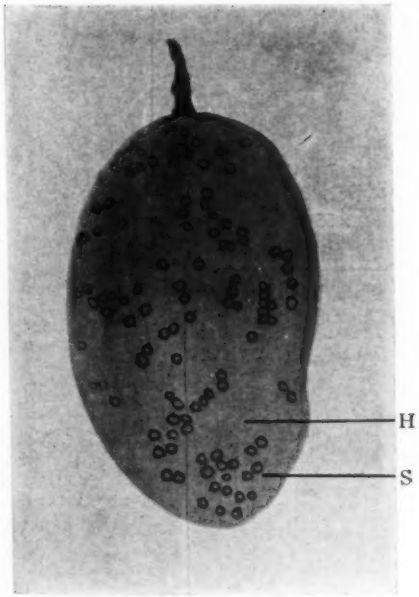
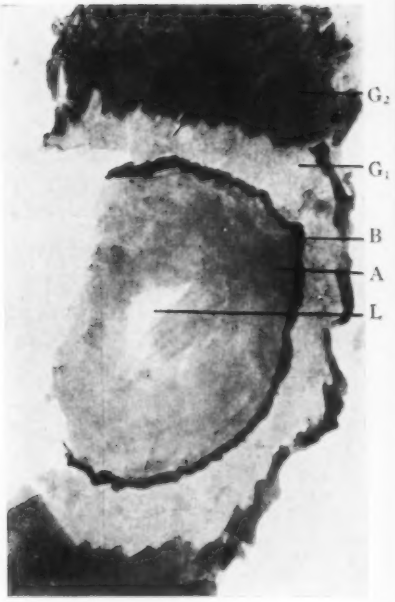


FIG. 3



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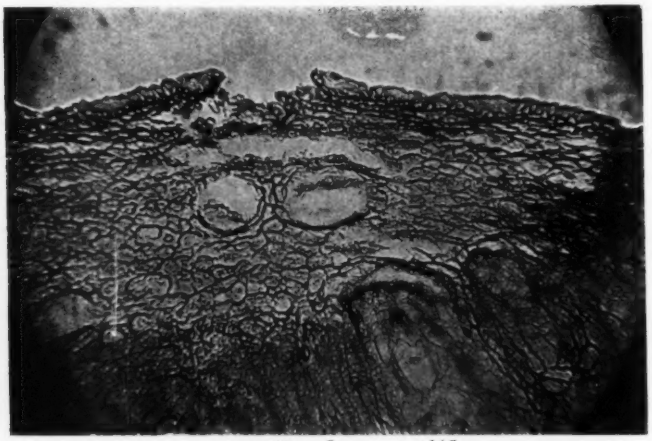

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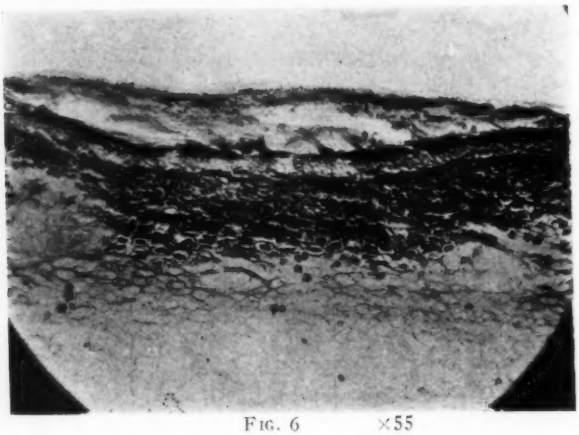

FIG. 6 ×55

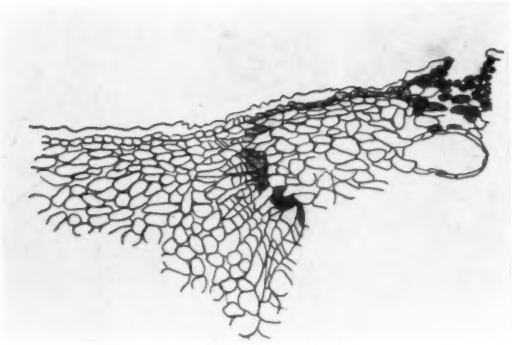

FIG. 7 ×168

PLATE III

FIG. 5. Section through a part of the tissue of a mango fruit fumigated for half an hour in 1·01% SO_2, showing (1) the affected region in a semicircle, the lenticel acting as the centre; (2) the early stage in the development of the cambium. × 115.

FIG. 6. Section through a part of the tissue of mango fruit fumigated with 2·3% SO_2 for half an hour showing (1) heavy deposits in the cells just outside the cambium and in the epidermis; (2) absence of starch grains in the cells of the affected region; (3) the suberisation of the new cells formed due to the activity of the cambium. × 55.

FIG. 7. Section through a part of the tissue of a mango fruit fumigated with 1·01% SO_2 for half an hour showing heavy deposits in the (1) epidermal cells, (2) the cells just outside the cambium. × 168.

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ERRATA

Proc. Ind. Acad. Sci., Vol. XII, No. 6, December 1940

Page 280, Table II. Third line—*for* "Acid soluble P"
read "Acid insoluble P".

and

" " Fourth line—*for* "Total acid insoluble P"
read "Total acid soluble P".

CONTRIBUTION TO THE CYTOLOGY OF *TRIDAX PROCUMBENS* LINN.

By Prof. T. S. Raghavan, M.A., Ph.D. (Lond)., F.L.S.
and
K. R. Venkatasubban, M.Sc.
(From the Department of Botany, Annamalai University)

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I. Introduction

This is one of the commonest weeds of the plains of South India. The generic name is an old Greek name used by Dioscorides meaning ' Summer Eating '—alluding to a plant that was a summer vegetable. There are about 27 Tropical American species. This species seems to have been introduced into this country from South America. There is no other species known in India.

The family Compositæ has been widely investigated by Cytologists and Geneticists. Though a number of genera have come in for a general or a casual investigation, a few like *Crepis*, *Dahlia* or *Chrysanthemum* have been very intensively worked upon. A number of workers have investigated almost all the aspects of *Crepis* and almost a complete phylogenetic history of the genus is now known. It has been established for instance that the genus *Crepis* is made up of three sub-genera, *Catonia*, *Eucrepis* and *Barkhausia*. They are characterised by progressively greater specialization and reduction in the length of the life-cycle. Diploid chromosome number 10 is considered to be the most primitive, although the most prevalent diploid number is eight. Secondary numbers ranging from 6–40 are known to have originated by interspecific hybridization and amphidiploidy, and polyploidy. Some process like reciprocal translocation must have led to reduction in the number from 10 to 8 and from 8 to 6. Segmental interchange between non-homologous chromosomes followed by meiotic irregularities must have been responsible for the elimination of one or more pairs of chromosomes.

All this was based upon intensive cytogenetical work of several years on more than a hundred species, obtained in a living condition from all parts of the world. Navashin (1929), Collins, Hollingshead and Avery (1929), Babcock and Clausen (1929), Babcock and Navashin (1930), Babcock and Cameron (1934) were some of the important contributors who were responsible for the correct understanding of the evolution in the genus *Crepis*. Similarly Lawrence (1929) has investigated the *Dahlias* and Shimotomai (1933 and 1937), the genus *Chrysanthemum*.

It is however curious, that in spite of their wide distribution in Tropical America, the genus *Tridax* should have escaped the attention of the American Cytologists. So far as we can make out, not even the chromosome number of any of the species has been determined. Our attention was attracted to this monospecific Indian representative, mainly on account of the scant attention that it has received at the hands of Cytologists; and in the present communication, the somatic and meiotic chromosome numbers have been reported for *Tridax procumbens* Linn. for the first time. Meiosis has been investigated in some detail, and a variety of abnormalities recorded. The development of the female gametophyte and of the embryo about which also there are no details available, will form the topic of another communication.

The heads in *Tridax procumbens* Linn. are heterogamous and are borne on long peduncles which may sometimes reach a length of even 2 ft. The ray florets are female with ligulate corolla. It is trifid and invariably pale

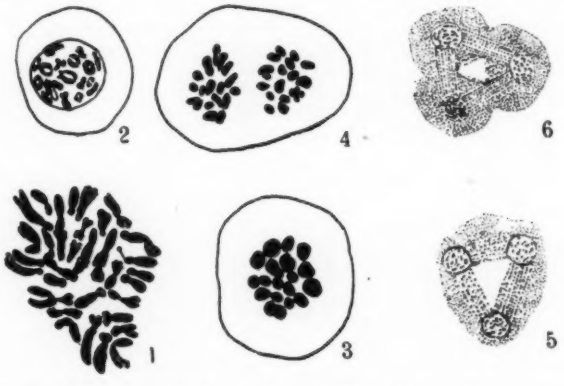
yellow in colour. However we were able to isolate from a natural population just half a dozen plants which possess ligulate flowers of a bright yellow colour. The cytology of this strain is being investigated. A number of heads from different localities in the University were examined to find out whether there is any constancy with regard to the number of ray and disc florets in individual heads. It was found that the range of variation in the ray-florets was from 4–7 while in the disc florets, it was 35–60.

II. Materials and Methods

The required plants were isolated from a wild population and grown in pots in the University Botanic Garden. Anthers were examined in aceto-carmine and then fixed in Navashin's fluid, after a pre-fixation in Carnoy's fluid. The usual paraffin method with chloroform as the solvent was followed. Sections which were cut at 10–18 microns were stained with Iodine-Gentian Violet. The same procedure was adopted with regard to the root tips also. Pre-fixation was found necessary and the most favourable time proved to be between 10 A.M. and 11 A.M.

III. Somatic Chromosomes

The diploid chromosome number is 36 (Text-fig. 1). On account of the large number and of the lack of prominent difference in the size of the chromosomes of the complement, no attempt was made at a quantitative idiogram analysis. All the chromosomes, however, possess centromeres of either the sub-terminal or of the median type. None has the terminal



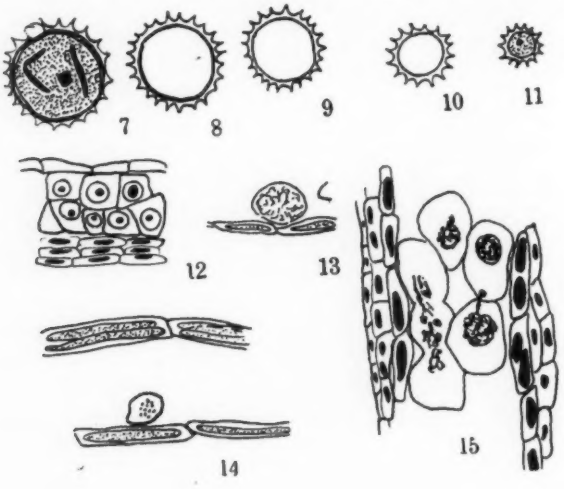
constriction. Only four pairs have the median type of centromere, the rest sub-terminal. There is a gradation in size in each of these two chromosome types. The longest median constricted and the longest sub-terminal type are of approximately the same length. So also with reference to the

shortest. Though a number of somatic plates were examined, it was found that only one sub-terminal chromosome possessed a satellite with a long trabant at its proximal end. The absence of satellite in its homologue cannot be easily explained. Whether it is a case of loss of the satellite brought about by elimination or only of the persistent non-showing of the other cannot be stated definitely.

IV. Meiosis

In the main, meiosis is regular. At diakinesis, eighteen bivalents are found, synapsis being complete (Text-fig. 2). A number of *p.m.c*'.s at this stage of development were examined and it was found that the majority of the bivalents were of the ring type. In a few cases as many as 12 rings and the rest rods, were observed. Text-fig. 3, and Plate V, Fig. 14, show first metaphase plates showing eighteen gemini. Text-fig. 4 shows on M II plate with the 18/18 distribution. Cross walls are formed only after the second division. This appears to take place by a process of furrowing, described further down.

The microspores that are rounded off by the process of furrowing are uni-nucleate to start with (Pl. V, Fig. 26). This divides into two, the tube nucleus and the generative nucleus (Pl. V, Fig. 21). Around the latter a distinct hyaloplasm is organised leaving no doubt of the fact that it is a cell and not merely a nucleus. The tube cell thus differentiated comes to envelope the generative cell (Pl. V, Fig. 22). The generative cell divides into two male cells. In Pl. V, Fig. 23, the two nuclei of the male cells are seen just below the tube nucleus whose nucleolus is seen very prominently. The male cells which are at first rounded become elongated very soon. This is the condition at the time of shedding (Pl. V, Fig. 24 and Text-fig. 7).



In spite of the prevailing regularity in meiosis, the pollen grains formed are of different sizes. Text-figs. 7–11 show their range in size. Notwithstanding this discrepancy they all appeared to be viable. Effort to germinate them as described by Wulff and Raghavan (1937) was not successful. But in a rough way viability can be ascertained by ordinary acetocarmine staining. When a pollen grain smear is made and stained with acetocarmine, only those which are viable take up the stain while there are others which do not take up the stain however much the staining may be prolonged. These are usually shrivelled and do not show out the conspicuous spinous wall (Pl. V, Fig. 27). Hence these may be regarded as sterile or inviable. Such an examination was made of a number of flowers and it was found that in addition to what may be tentatively regarded on the basis of their taking up the stain as viable grains, there were a variable number which would not take up the stain and which on this account, must be regarded as sterile. The following table gives an idea of the proportion of the fertile and sterile grains:

TABLE I

Plant No.	Flower No. on the same head	Total No. of pollen grains	Fertile grains	Sterile grains	Percentage of fertile grains
1	*a*	239	159	80	66
	b	239	146	93	61
2	*a*	207	147	60	71
	b	179	96	83	53
3	*a*	375	225	150	60
4	*a*	205	165	40	80
	b	261	161	100	54
5	*a*	239	159	80	66
	b	239	146	93	61

It can be seen from the above table that the percentage of viable pollen grains is between 50–80 and such wide fluctutation is found not only in flowers belonging to different plants but also in the flowers borne on the same head. This is the case in 2 *a* and *b* and 4 *a* and *b*. The interesting thing about *Tridax* appears to be that the fertile grains or at any rate the

grains possessing normal visual viability are of different sizes. Text-figs. 7–11 show them all under the same maginfication.

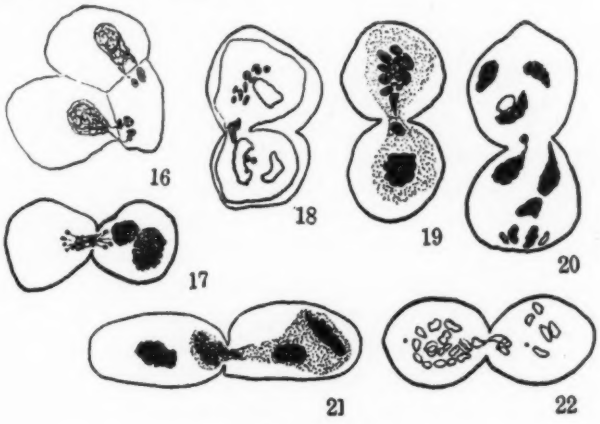
The pollen grains are rounded off by the process of furrowing and not by the regular formation of cell plate. Text-fig. 6 shows the three-furrowed state. Wodehouse (1921) considers that the prevailingly three-furrowed (tricolpate) type of pollen grain is directly associated with the tetrahedral arrangement of the four microspores in the *p.m.c.*, which is the usual arrangement in the Dicotyledons and that other numerical configurations of furrows are associated with other arrangements in the tetrad. This prevailingly tetrahedral arrangement of pollen tetrads is due to the ever present tendency among cells to assume least surface configurations.

The first division offers nothing unusual. As the daughter nuclei enter upon the telophase and reorganize, the fibres become numerous. These initial stages in phragmoplastic activity persists for some time. The fibres show some thickening midway between the two daughter nuclei as if about to form a cell plate, but ultimately the phragmoplast disappears without completing cell division.

In this species the second division usually takes place in such a manner that their axes lie in planes at right angles to each other. As the chromosomes separate and move towards the poles of the spindles, the connecting fibres are straight and about this time four further spindles appear between the four nuclei (there could only be seen three nuclei and three spindles due to the tetrahedral arrangement) of the second division. Thus the *Tridax* tetrad is provided with six connecting spindles (Text-fig. 5). Each nucleus including the one which cannot be seen in a focus with the upper three comes to possess ultimately three radiating spindles.

The exact manner in which these additional spindles arise does not seem to be very clear. Devisé (1921) suggests that they may be regarded as the reappearance of the heterotypic spindle split longitudinally into two halves. Soon after the appearance of the added spindles in the tetrad, quadripartition of the cell begins. This is accomplished by the process of simultaneous furrowing essentially as described by Farr (1916) for the tetrads of *Nicotiana*. It is as though each nucleus were a centre of attraction about which the cytoplasmic mass tends to round up. The furrows appear as two and three cornered depressions and growing inwardly they finally meet in the centre forming a three-cornered hole (vacuole) through the centre of the tetrad (Text-fig. 6). This leaves each nucleus in its own cell but connected by connection with its adjacent neighbour of the tetrad, and each of these channels encloses the remains of the poorly developed connecting spindle or

phragmoplast. At the same time the perpheral surface of the cell is cut by four furrows, one between each pair of nuclei and opposite to each of these corners of the central opening. By manipulating the focus of the microscope these furrows are found to be annular constrictions encircling the pit connections. These constrictions become deeper and ultimately cut through between the daughter nuclei and thus separate the daughter cells. Upon their separation, the daughter cells lose all traces of the pit connection and the four cells flatten slightly against each other tending to conform to the general curvature of the group as a whole. At this stage the spermocytes are smooth walled Their walls soon begin to thicken and the spines appear as papillæ. They become more turgid and rounded and then the germinal furrows become evident. It was said that the viable (stain-taking) pollen grains ranged in size from the normal to the very small (Pl. V, Figs. 25 and 26). To our mind, while the small grains are formed in a manner described further down, the others showing only slight variation (Pl. V, Fig. 27) in size are to be considered to have been normally formed. The very nature of their origin by the method of furrowing is probably responsible for the slight difference in their diameter.



V. The Tapetum

The behaviour of the tapetal cells calls for some mention. There are usually three layers of parietal cells of which the first one beginning with the anther sac is the tapetum. Initially all the three layers are of equal size and form (Text-fig. 12), *i.e.*, when the microsporogenous cells have not yet entered upon their meiotic phase. As the latter progresses, the tapetal cells show a slight enlargement. There is however a very marked and almost sudden growth between zygotene and M I (*cf.*, Text-figs. 13 and 14). This growth

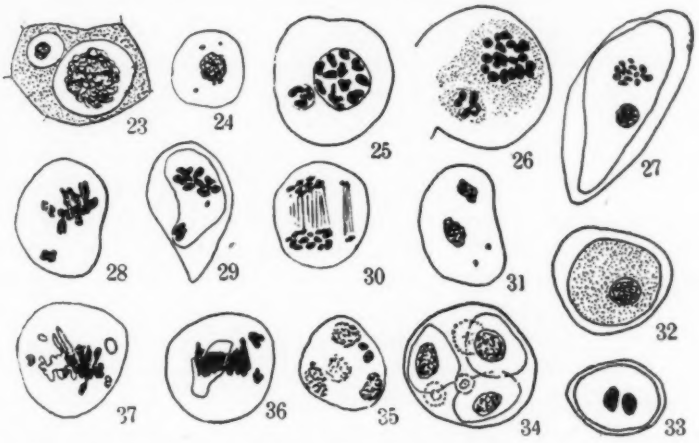
period is marked by an intense mitotic activity in the tapetal cells. Tapetal cells containing one nucleus change this condition and come to possess two and four nuclei (Text-fig. 15). But seldom do they remain separate, fusion taking place immediately. But more frequently, and this is almost the prevailing condition, the division of the tapetal nucleus does not result in the organization of a definite daughter nucleus as such, but there is a multiplication of the chromosomes without any anaphasic separation. The result is, long cylindrical nuclei are formed many times the length and diameter of the *p.m.c.* (Pl. IV, Figs. 5 and 6). Compared to the growth of the tapetal nuclei those of the *p.m.c.* is insignificant. In some two such long nuclei are seen and in others only one. The original single nucleus without attaining the binucleate condition goes on in some cases, dividing mitotically without chromosomal separation and thus result in single long tapetal nuclei. In others it would appear this is resorted to after the two tapetal nuclei have been organized (Text-fig. 14). There would appear, however, no correlation between such a behaviour and the position of the tapetal cells relative to that of the anther sac. But it was found that the position of the tapetum beginning with the inner side of the sac, showed more frequently the presence of the two long nuclei. The tapetal nuclei are discharged into the cavity and the disintegration of the nuclear matter takes place in the usual manner.

VI. Meiotic Aberrations

(*a*) *Cytomyxis.*—Though meiosis was in the main normal there were frequent cases of abnormalities of many kinds. These occurred promiscuously and bear no relation, so far as our observations went, to any particular location of the anther sac or of the flower on the head. It is therefore impossible to explain these on physiological grounds.

The most frequent of these, was the phenomenon of Cytomyxis and the most interesting fact about this is that it occurs in almost all stages of meiosis. It is very wide-spread in occurrence also. There was not a loculus but exhibited cytomyxis at some stage or other of the *p.m.c.* Text-figs. 16 to 22 show cytomyxis at different stages. Fig. 16 in prophase; Figs. 22 and 19 in diakinesis and prometaphase respectively. The transference of nuclear matter may be whole or partial, more frequently it is of the latter type. In Text-fig. 17 there is evidence of entire migration of prophase nucleus while in Fig. 19 there is only partial migration. In Pl. IV, Fig. 4, there is complete migration of early prophase nucleus. In Fig. 18 we see a portion of A I passing into the adjacent cell while in Fig. 21 there is complete migration of an entire nucleus. In Fig. 20 there are shown two *p.m.c.*'s with an opening between them through which partial cytomyxis is taking

place. The two *p.m.c.*'s are in the second telophase; but in the lower cell, one of the telophase groups has divided further to form groups of their own so that in that cell instead of four telophase groups there are seven.



(*b*) *Binucleate pollen mother cells.*—Normally *p.m.c.* is uninucleate (Text-fig. 32), but very commonly pollen mother cells were observed which contained either two large normal sized nuclei (Text-fig. 33) or one large and one small nucleus (Text-fig. 23). The latter condition however was more common. Evidence at later stages of meiosis reveals that in both types of syndiploidy, *i.e.*, (1) the occurrence of normal nuclei in pollen mother cell and (2) the occurrence of one normal and one small, the nuclei behave independently throughout meiosis. Figs. 23–37 show all stages of meiosis undergone by the two nuclei of the *p.m.c.*, one big and one small, just as if they were two independent *p.m.c.*'s. Pl. IV, Figs. 1–3, show photomicrographs of this simultaneous division of the big and small nuclei at resting stage, diakinesis and M II respectively. It will be seen from these figures that in the nuclei, the division stages are entered upon simultaneously. This simultaneous independent behaviour of the two nuclei, though almost the rule is sometimes departed from, when we find that as for instance in Text-fig. 27 and Pl. V, Fig. 15, while the bigger nucleus is in M I, the smaller nucleus is in its resting condition.

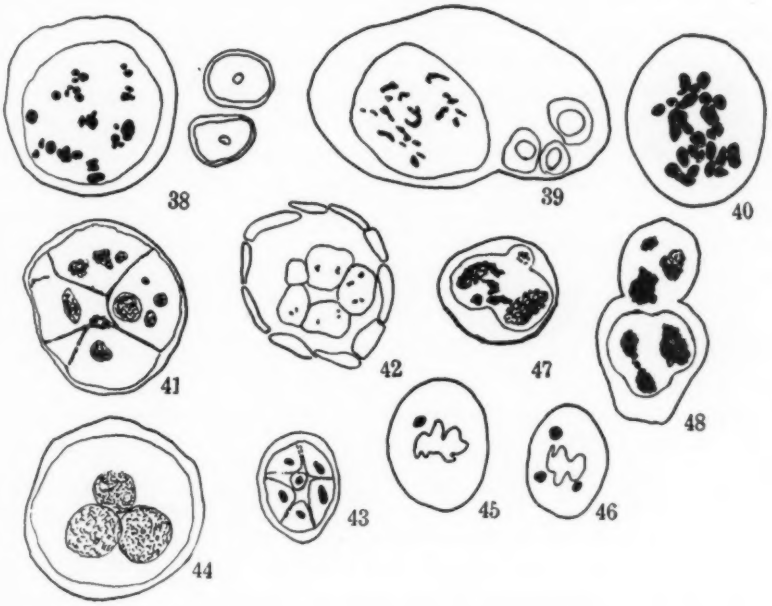
This independent behaviour normally results in the formation of four haploid and four sub-haploid pollen grains. These are the small grains referred to previously, and which by taking up the stain showed that they were viable. In the case of the other type, the occurrence of two normal-sized nuclei, eight haploid grains would result. The most common

condition in the former type is the formation of four haploid grains and only two sub-haploid grains (Text-figs. 34 and 35). This means that in such types, the second division of the smaller nuclei is suppressed. In Pl. V, Figs. 25 and 26, are seen some of these sub-haploid pollen grains arisen in this manner. They are not shrivelled as the others formed by eliminated chromosomes are.

The *p.m.c.*'s containing two normal nuclei have presumably originated by suppression of wall formation at the premeiotic mitosis. But the origin of the small nuclei in the *p.m.c.*'s is not quite clear. Two explanations may be offered; the first is that it may be a case of elimination of chromosomes. Such an extrusion is quite common in hybrids, triploids and also in X-rayed progeny. But in all these micro-nuclei or micro-cells, as described by us (Raghavan and Venkatasubban, 1940 *a*) in triploid *Urginea* result, and these seldom round off as pollen grains. Both the nuclei will be deficient and it is no wonder that neither of them functioned. Nor is it common to find these eliminated chromosomes undergoing the full round of meiotic process, right from the resting stage, to the organization of the grains; this would imply not the elimination of one or more chromosomes but the abstriction of a portion of the resting stage nucleus of the *p.m.c.* Normally extrusion takes place at M I, A I, and M II, and the eliminated chromosomes constitute themselves into one or more micro-nuclei and there they end. Such an elimination also occurs and it is these that constitute the shrivelled up bodies intermixed with the pollen grains. But the totally different behaviour of these small nuclei described herein, shows that these are not to be regarded as cases of eliminated chromosomes. Abnormal spindle behaviour at premeiotic mitosis of the *p.m.c.* must therefore be considered as the second and the more likely cause of the origin of the small nuclei. It may be that there was a failure of co-ordination of one of the spindles at the premeiotic mitosis. These abnormalities are due more to somatic instability than to any defect in the process of meiosis itself.

(*c*) *Fusion of pollen mother cells.*—*P.m.c*'s showed a strong tendency to fuse with one another. Four or more *p.m.c.*'s aggregated and fused, the cross walls were absorbed and each loculus came to possess frequently only one such fusion product (synpollen mother cell), if such an expression could be applied. Text-figs. 42 and 43 show five and six *p.m.c.'s* coming very close together in an anther loculus. The next stage of fusion is shown in Fig. 41, and Pl. IV, Fig. 9. Frequently the nuclei of the fusin *p.m.c.*'s show early signs of degeneration, by breaking into unequal bits of chromatin masses (Text-figs. 41 and 42). But sometimes they seemed to enter upon further meiotic activity. In Text-figs. 38 and 39, and P. IV, Figs. 7 and 10

we see the resolution of the chromosome like bodies. Undoubtedly all the chromosomes are not present, as at least some of the fusing nuclei must have degenerated. This disparity in size between a synpollen mother cell which may now be regarded as a giant pollen grain in the making, and the grains formed out of normal meiosis is shown in Text-figs. 38 and 39. But these synpollen mother cells never succeed in forming a giant grain. They degenerate sooner or later.



Fusion of *p.m.c.*'s is one of the important methods in the formation of polyploid gametes. No viable polyploid gametes were however found in this case. Text-fig. 44 (Pl. IV, Fig. 8) is hard to interpret. It may be a case of fusion of three *p.m.c.*'s at the resting condition of their nuclei. The fusion has taken place so completely that not even vestiges of the *p.m.c.* walls are visible as in the other cases (*cf.* with Figs. 41 and 42) or it may be something like the binucleate *p.m.c.* described previously. Just as we get two normal-sized nuclei in a *p.m.c.* owing to failure of wall formation at the premeiotic mitosis, we may also get the trinucleate condition where one of the nuclei has again divided unaccompanied by wall formation. Or it may be a case of cytomyxis; but the relatively large size of the cell precludes the latter possibility. The first method seems to be the most likely in the matter of its

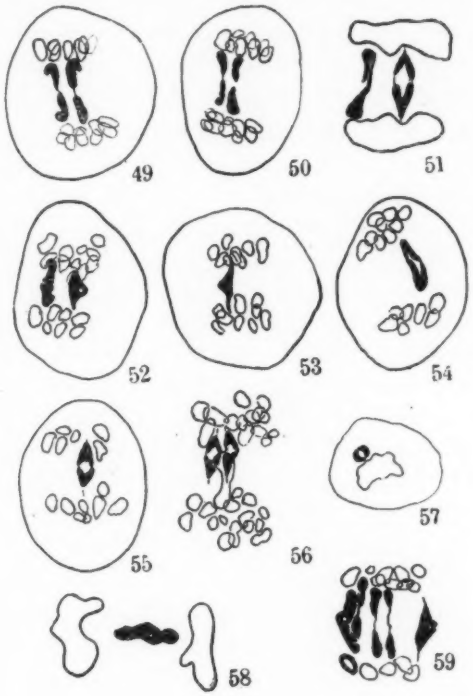
formation. All these three methods would result in the ultimate formation of polyploid microspores.

(*d*) *Semiheterotypic division.*—Frequently chromosomes at first division are widely scattered over the spindle (Text-fig. 40). At a stage corresponding to the anaphase the chromosomes were still widely scattered on the spindle as laggards. These were so numerous that the nuclear membranes normally laid down at the poles instead of cutting through the spindle-figure inclosed the entire chromosome complement in a single nuclear membrane. The single nucleus thus formed was often dumbell-shaped and gave the appearance of an amitotic division. In many instances however the nucleus was rounded out to a normal shape although it contained the somatic number of chromosomes. These may be regarded as restitution nuclei. Those of normal shape must be expected to undergo normal division resulting in the formation of a dyad of nuclei each of which is diploid. Such a behaviour was designated semi-heterotypic division by Rosenberg (1917). The semiheterotypic division is of wide-spread occurrence in known and suspected hybrids.

(*e*) *Extrusion, non-disjunction, Bridge formation, etc.*—Cases of extrusion of chromosomes either univalents or bivalents were also met with. In Text-fig. 57, Pl. V, Fig. 13, a bivalent is seen off the first metaphase spindle. In Text-figs. 45 and 46 we see one and three univalents respectively extruded off the spindle. This means that pairing was not complete. Though in the majority of cases complete synapsis was the rule, in some a few chromosomes would not pair. These univalents were extruded. How far this is due to hybridity must for the present remain an open question. Text-figs. 54–56 are clear cases of non-disjunction. The bivalents in these are seen to reach the pole without disjoining. These cases are peculiar in that the bivalents in question act as laggards. They do not seem to reach the poles earlier than the rest as the other case (Text-fig. 57 and Pl. V, Fig. 13) of the extruded bivalent would indicate. The bivalents may not be included in the daughter nuclei in which case like the eliminated univalents they may also organise micronuclei. If however they are included in the pole they would bring about a disparity in the number of chromosomes at each end. Such a disparity was not infrequent.

Frequently chromatin masses suffered fragmentation into bits of unequal size. Soon after the first division, the telophase nuclei so organized, showed signs of division in this what may be regarded as an amitotic manner. Text-figs. 47 and 48 and Pl. V, Figs. 11 and 16, show such telophase masses. These of course, are symptoms of degeneration.

Another frequently occurring phenomenon is that of the stretching of the chromosomes along the spindle during the first division to form bridge-like configurations. These are not considered to be inversion bridges mainly



because of the absence of fragments associated therewith. Most of these are due to delayed disjunction (Text-figs. 49–51). In others there is delayed terminalization of the chiasmata and the bivalents lie on the spindle (Text-figs. 52 and 53). Text-fig. 58 and Pl. V, Figs. 17 and 18, are presumably trivalents lagging on the spindle. In Text-fig. 59 and Pl. V, Fig. 19, a number of bridges are stretched along the spindle.

VII. Discussion

(*a*) *The balance system of meiosis.*—In a recent paper, Gentcheff and Gustafsson (1940) have recorded what may be regarded as meiotic abnormalities in some apomictic species of *Hieracium*. Not only is it a related genus to *Tridax*, but some of the abnormalities, recorded therein, conform to those described in this paper. It may be useful to discuss whether or not their interpretation is applicable to this case. Gentcheff (1937) first found

a peculiar mode of tapetum development in several apomicts of *Hieracium*. This kind of tapetum development and degeneration was considered by him peculiar to apomicts. Since pollen production in most apomictic Archieracium biotypes is very low, and tapetum degeneration differs from the behaviour in sexual types, it was suspected that some casual connection existed between these. This led to a detailed re-examination of tapetum development, and meiosis in *Hieracium robustum*.

This is an apomict, never producing pollen; the meiotic irregularities are classified into three broad types and all these three are connected in some way with the tapetum development.

In division type 1, a sort of a semi-heterotypic division (Rosenberg, 1927) is seen. There is no pairing. Univalents are scattered over the metaphase spindle. Second division is also common. Some of these have also been recorded here. Anaphases are irregular leading to the origin of poly-nuclear pollen mother cells. In this type, division starts when tapetal cells are uninucleate and very small, and pollen mother cells are very small also. Prophase is remarkably precocious. In some cases metaphases contain mitosis-like chromosomes and more or less pseudohomotypic (Gustafsson, 1935) in character with no chromosome pairing and univalents gathering in the equatorial plane and dividing at first division and with no second division.

In division type 2, double reproduction of chromosomes takes place. In this the cells and nuclei are reported to have grown intensely. In response to the changed proportion of cell and nucleus volume, the chromosomes are forced to reproduce twice.

Division type 3 is associated with bivalent and fragmentation phenomena. Prophases and metaphases of this type of division occur at very advanced tapetum stages, even more advanced than in type 2. Four-fused and eight-fused tapetal cells are very common. The number of bivalents is variable. Anaphase separation is irregular, due to the high number of univalents. Inversion bridges are frequent at I and II divisions. In the same plate three or four bridges may be found indicating a high degree of structural differences. The most conspicuous feature in the case of bivalent formation is the extreme fragmentation of chromosomes.

From these and other data it is concluded that bivalent formation occurs exclusively when a proper balance exists between tapetal growth and activity, pollen mother cell growth, prophase onset and chromosome reproduction. They emphasise the view that meiosis is greatly under the physiological influence from or *via* the tapetum cells. This would appear to be especially note-

worthy in organisms where pollen mother cells form one cell-row only, covered with tapetum cells on all sides. For example, in a *Crepis* apomict Stebbins and Jenkins (1939) found a correlation between the mitotically inactive tapetal cells and the undeveloped pollen mother cells. Bivalent formation occurs exclusively when a proper balance exists between tapetal growth and pollen mother cell growth.

For all practical purposes, *Tridax* is a sexual diploid. Whether it is a secondary polyploid, as it may well turn out to be, can be ascertained only after we have some knowledge of the basic chromosome number. Of this we have no data at present. The first point to be noted is that some of the meiotic aberrations, like semi-heterotypic division, pseudo-homotypic division, which have been known to occur in apomicts, are common in sexual species also. As far as our observations go, no exact connection exists between the occurrence of the aberrations and the position of the flowers on the head. Their occurrence is more or less at random, and apparently not governed by any physiological factor, as determined by a particular location on the inflorescence. Intermixed in the same loculus with pollen mother cells showing regular meiosis, the ones showing semi-heterotypic division also occur. The behaviour of the tapetum as described in the text is more or less uniform, and obviously on account of the very nature of the distribution of the abnormal pollen mother cells, no correlation could subsist between the abnormalities and the behaviour of the tapetal cells.

Of the three metaphase types of meiosis, type I was found here and there. We were not able to find any case of double reproduction of chromosomes, giving rise to an increase in the chromosome number at interphase. Of the third, there were cases of bridge-formation. But so far as our observations went most of them were due to delayed disjunction and not inversion bridges. No fragments could be seen. Gentcheff and Gustafsson (1940) described a veriety of methods of fragmentation but no evidence of fragmentation existed in our material. Fig. 38, 39 may at first suggest the presence of fragments but the cell-size and the other stages described show unmistakably that it is the fusion product of a few pollen mother cells in which the chromatin masses have undergone contraction and partial regeneration.

The interesting feature is that in some details the meiotic behaviour of the pollen mother cells in the present material is identical with that of a apomictic forms. It is mainly upon a study of these apomictic species that the correlation was established between the pollen mother cell behaviour and that of the tapetal cells. If that is so, either of two alternative inferences

seem justifiable. That no such definite correlation is tenable at least so far as this genus is concerned or since these are almost characteristic of apomictic forms, *Tridax* must be regarded as an apomict. Seed production however is quite copious and that is about the only method of propagation known in this species. That the meiotic peculiarity could in any way be connected with any abnormality in seed formation—whether it could be a case of agamospermy—seems quite unlikely.

(*b*) *Cytomyxis.*—This phenomenon was first described by Gates (1911) in *Oenothera gigas* when he applied this term to the process of extrusion of chromatin from the nucleus of one pollen mother cell to that of an adjacent cell. Since then the phenomenon has been reported in a number of genera. It has been noted especially in cytological description of grasses (Church, 1929 and 1936). In the grasses different types of cytomyxis have been reported. In the first type, the phenomenon was seen only in the spireme stage, but with resultant extrusions persisting in later stages. In the second type the process was manifested in diakinesis. It was also observed that the greatest amount of cytomyxis is correlated with the greatest amount of irregularities in the maturation divisions. In the third type chromatin transference was observed during interkinesis.

In the present material cytomyxis has been observed in almost all stages of meiosis and of all the meiotic abnormalities this is the most frequent so far as this material is concerned. If one made an analytical study of the plants in which phenomenon has been recorded so far, two facts will stand out prominently. Firstly that in all plants there is a certain amount of sterility, so that it is almost certain that cytomyxis is responsible for the degeneration of the pollen mother cells and even of whole anther sacs. To this is presumably due the not insignificant proportion of sterility that is so common a feature of the pollen grains of this species (*cf.* the table giving the percentage of sterility). Secondly cytomyxis recognised as an abnormality has been reported in several definitely known hybrids. At spireme stage it has been seen in *Oenothera Rubrinervis* (Gates, 1905). A hybrid form of *Typha angustifolia* showed the phenomenon at diakinesis (Roscoe, 1927). Kattermann (1933) described it in the *p.m.c.*'s of *Triticum* × *Secale* hybrids and Percival (1930) in the hybrids of *Aegilops* sp. × *Wheats*. So rather than regard it as an artefact as Sinoto (1922) would have us believe, its occurrence is undoubtedly associated with hybrids. In the investigation of Church (1929), he found that while the occurrence of this phenomenon was very striking in connection with hybrids the examination of considerable material of *Phalaris arundanacea*, a normal diploid, revealed no cytomyxis.

(*c*) *Non-disjunction.*—Cases of non-disjunction in the heterotypic division have also been met with as illustrated by figures. This is the particular type of unequal chromatin distribution in which one or two bivalents may approach the equatorial plate, but are pulled undivided to one of the poles. Consequently instead of the usual 18/18 segregation, 20/16 and 22/14 in anaphase were often seen.

Non-disjunction has been reported frequently in hybrids. *Oenothera lamarckiana* may show an anaphase segregation of 8/6 or 9/5 instead of the usual 7/7 (Sinoto, 1922). In the tetraploid species of *Datura*, segregates of 23/25 instead of the 24/24 are quite regularly found. The same situation has been reported in *Nicotiana alata* (Ruttle, 1927). Unequal segregation leading to the formation of pollen grains of varying number of chromosomes (17–29) instead of 23, has been reported in *Scilla indica* (Raghavan and Venkatasubban, 1939).

When gametes of different numerical chromosome equipment mature as a result of unequal distribution or non-disjunction, it is obvious that they are functional in producing dysploid offspring. This particular type of polyploidy has been seen in a striking manner in the dysploid series of *Carex* (Heilborn, 1924).

(*d*) *Irregularities in Nuclear Divisions—their causes.*—Meiotic irregularities are, as shown already, widely prevalent in species hybrids. They can also be caused by physical or chemical agents. According to Kostoff (1930) one has to take into consideration bio-physical and bio-chemical phenomena involved in the cells elsewhere accompanied by irregular nuclear division. The direct activity of the physical or chemical agents is expressed by change in the viscosity of the cytoplasm (CV). An increase or a decrease might lead to a reversible or irreversible coagulation. The latter means death to the cell. The determination of the relative CV has been made by Brownian movement, cytoplasmic streaming, centrifuging and plasmolytic methods. Various agencies have been found to affect the CV. By wounding, coagulation and an increase of CV occurs in the neighbouring tissue around the wound. A slight increase of CV offers favourable conditions for bringing about cell divisions. Apparently such a phenomenon occurs in galls, tumours, etc. The foreign substances penetrating into the dividing tissue increase the CV and this is followed by intensive cell division. Even in normal cell division at the beginning of the anaphase, there is according to Chambers (1919), a decrease of CV. The occurrence of this diminution is prevented in galls, tumours, tapetum, etc., by a continuous activity of the foreign agents. If the CV is held relatively high by these, chromosome division occurs,

separation being inhibited by the high CV, and somatic polyploidy is the result. Multinucleate cells originate when the difference between the normal CV during the anaphase and the one maintained by the activity of these foreign bodies is not very great, and only a slight separation of the chromosomes takes place. If this difference is relatively small non-disjunctions occur and chromosomes lag on the spindle. Other agencies that cause a similar change in CV and consequent dislocation of nuclear divisions are X-rays, Radium rays, Ultra Violet rays of 280 μ wave-length and various chemicals. In a previous communication (Raghavan and Venkatasubban, 1940 *b*) we have described some of the meiotic abnormalities induced by X-radiation and also by colchicine.

The first question that arises from the preceding discussion is whether the irregularities of nuclear and cell division in species hybrids are controlled by causes similar to those controlling irregularities springing from the above mentioned causes. This is answered by Kostoff (1930) in the affirmative. The germ plasm of one species of the hybrid contributes something foreign to that of the other. That which is foreign causes physical and chemical changes in the protoplasmic properties of F_1 including the CV. The lagging chromosomes and the occurrence of somatic polyploidy so common in hybrids are a result of increase in CV. The effect on *p.m.c.*'s is even greater. In a similar manner X-rays cause an increase in CV and chromosomal aberrants are common among the progenies of X-rayed plants, undoubtedly as a result of disturbance in the chromosome distribution in the X-rayed plants.

The second question is to find an explanation of the peculiar behaviour of the tapetal cells. In the light of the preceding discussion the morphology of the tapetum appears to be an inevitable result of the effect of special agents coming from the *p.m.c.* These agents may be considered foreign for the soma and raise the CV of the tapetal cells so that a continuous chromosome division without separation takes place. Plurinucleate tapetal cells would appear to be widespread among plant species. The binucleate condition prior to disorganization is almost the rule. Uninucleate tapetal cells at the time of their disintegration is the least common. A natural question is, are all cases of multinucleate tapetal cells to be regarded as species hybrids, even though they may not exhibit any other visible hybrid characters? Such an inference would appear warranted if we accepted *in toto* the principles underlying the preceding discussion. To our mind it seems that it must await further correlated study, establishing a definite relationship between the tapetal behaviour and the hybrid nature. Multinucleate tapetal cells and somatic polyploidy appear to be common in species hybrids

but such a behaviour is also exhibited by species which have not yet been proved to be hybrids or whose hybrid nature is not yet established beyond doubt. How far this is applicable to cases of structural hybridity must for the present remain unanswered. The other alternative is, that whether a species is a hybrid or not, the special agents that come from the *p.m.c.* act as foreign bodies and raise the CV of the tapetal cells, and this leads to the consequential mitotic aberrations. If we accept the first alternative then it follows that inasmuch as in hybrids meiotic aberrations such as those described are very common, the tapetal behaviour and these meiotic irregularities must be considered as correlated. In other words multinucleate tapetal cells, somatic polyploidy of the tapetum, etc., must be expected only in such species which being species hybrids would also show aberrations in the meiotic divisions of the pollen mother cells.

This however is not substantiated by facts, for such a tapetal behaviour as is said to be universal in hybrids, occurs unassociated with any meiotic irregularity in many cases. We have found recently (Raghavan and Venkatasubban, 1940 *c*) in a species of *Crescentia* a similar plurinucleate condition of the tapetum but there is absolutely nothing abnormal in respect of the meiosis of the *p.m.c.*

It seems probable therefore that if the tapetal behaviour must be explained on the basis of the increased CV, then the second alternative must be regarded as being more tenable. So far as the species under investigation is concerned, it seems safe to say that the occasional prevalence of the meiotic abnormalities may be taken as a fair indication of the contribution of some amount of ' foreign blood ' in the make up of the species. This is in a way supported, as has already been mentioned, by the widespread occurrence of the phenomenon of cytomyxis. The suggestion made in the earlier part of the discussion that there appears to be no correlation between meiotic abnormality such as semi-heterotypic division, etc., and the peculiar tapetal behaviour, seems to be justified from this point of view also.

(*e*) *Tridax procumbens—a true breeding hybrid*?—From the foregoing description of the meiotic process in the species and of the behaviour of the cells of the tapetum, one thing stands out very prominently. And that is, it exhibits a number of meiotic features which are usually associated with hybrids and most of these what may be regarded as meiotic abnormalities have to do with the ultimate production of polyploid gametes. Such are, fusion of *p.m.c.*'s cytomyxis, suppression of wall formation at premeiotic mitosis, restitution nucleus formation, etc. But the interesting thing is

that though these phenomena are exhibited in their earlier stages, the respective processes are not carried through to their ultimate end, namely to the formation of polyploid grains. Degeneration sets in sooner or later. These facts set one thinking whether *Tridax* is a true breeding hybrid. Hybridity associated with polyploidy undoubtedly plays an important part, as recent investigations have shown, in the evolution of plant species. Polyploid gametes have been produced as a result of hybridization. These gametes have proved viable in various combinations and have resulted in the production of polyploid hybrid forms. These hybrids intercrossed or back-crossed have led to the establishment of stable types. Such stable types exhibit usually a different chromosome number from either of the parents and the reduction divisions are regular or nearly so. Certain of these polyploid forms are known to be even more fertile than the diploid forms. Having these facts in our mind, it may occur to one that in the evolution of this species, some of these phenomena have been involved. The occurrence of the meiotic aberrations usually associated with hybrids, but which do not either lead to the ultimate formation of polyploid gametes or affect in the least the fertility and seed-production of the species, seem to imply to our mind that hybridity has played an important part in the formation of this species. We are not unaware, however, of the difficulties lying in the way of such a concept, especially when it is known that *Tridax procumbens* is an exotic species and that in India it is monospecific.

VIII. Summary

The diploid and haploid chromosome numbers of *Tridax procumbens* Linn., have been determined for the first time to be 36 and 18 respectively.

Meiosis has been described in detail.

A number of phenomena in the nature of (*a*) fusion of *p.m.c.*'s, (*b*) semi-heterotypic division, (*c*) cytomyxis, (*d*) binucleate *p.m.c.*'s are exhibited which instead of culminating as they should, in the ultimate formation of polyploid gametes, abort somewhere near the end of the respective processes.

A number of other things like delayed-separation-bridges, non-disjunction, amitotic division of the T I nuclei are also of frequent occurrences.

It is suggested that in the evolution of this species, hybridization has played an important part.

The behaviour of the tapetal nuclei is described and found to be somewhat like that in Apomicts and hybrids. The view that meiotic aberrations such as occur in these forms are correlated to the peculiar behaviour of the tapetal cells is discussed and not accepted.

The bio-physical and bio-chemical phenomena involved in irregularities of nuclear and cell division is discussed and the tapetal behaviour explained on this basis.

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EXPLANATION OF PLATES AND TEXT-FIGURES

Plates IV and V are photomicrographs.

The Text-figures were drawn at table level with an Abbe drawing apparatus at bench level; their approximate magnifications are given against each.

PLATE IV

FIGS. 1–3. *P.M.C.* with one large and one small nuclei, showing simultaneous division stages, early prophase, diakinesis, M I and M II.

FIG. 4. Complete cytomyxis at prophase; same as Text-fig. 17.

FIGS. 5, 6. Tapetal nuclei, greatly elongated, due to repeated division unaccompanied by separation.

FIGS. 7, 10. Syn-*p.m.c.* simultating to enter upon division stages.

FIG. 8. Trinucleate *p.m.c.*; same as Text-fig. 44.

„ 9. An earlier stage in the fusion of the *p.m.c.* Note the remains of the walls.

PLATE V

FIG. 11. Amitotic division of the telophase nucleus (same as Text-fig. 48).
„ 12. Bridge in the spindle.
„ 13. Bivalent off the spindle.
„ 14. *P.M.C.* M I, 18 bivalents.
„ 15. Binucleate *p.m.c.*—non-simultaneous behaviour.
„ 16. An earlier stage than Fig. 11.
FIGS. 17, 18. Trivalent lagging on the spindle.
FIG. 19. Multiple bridge—delayed disjunction.
„ 20. Non-disjunction.
„ 21. Binucleate pollen grain.
„ 22. Generative cell organised.
„ 23. Generative cell divided into two male cells.
„ 24. The male cells assume an elongated form (same as Text-fig. 7).
FIGS. 25, 26. Pollen grains, showing the very small ones intermixed with the normal ones. These appear viable and have arisen by the independent behaviour of the small nucleus of the binucleate *p.m.c.* Note also the presence of shrivelled grains.
FIG. 27. Pollen grains of slightly different sizes due to the " furrowing origin ".

LEGEND TO TEXT-FIGURES

FIGS. 1–6

FIGS. 1 and 4 × 3,600. FIGS. 2 and 3 × 2,700. FIGS. 5 and 6 × 1800.

FIG. 1. Somatic metaphase plate; 2*n* 36. Note the satellited chromosome
„ 2. *P.M.C.* in diakinesis.
„ 3. *P.M.C.* M I.
„ 4. *P.M.C.* M II. Showing 18/18 distribution.
FIGS. 5, 6. Furrowed origin of the pollen grains. ca. 180

FIGS. 7–15. × ca 900

FIGS. 7–11. Pollen grains of different sizes; 11 has arisen from the small nuclei of the binucleate *P.M.C.*
„ 12–15. Tapetal behaviour at different stages of the *p.m.c.*

FIGS. 16–22. × ca 1800

Cytomyxis at different stages of meiosis.

FIGS. 23–37. × ca 1800, except FIG. 27 which is × ca 900

FIG. 23. Binucleate *p.m.c.* nuclei of different sizes; same as Plate VI, Fig. 1.
FIGS. 24–26, 28–31, 34–37. Showing different stages of the simultaneous division of the two nuclei. In 34 and 35 we see 4 haploid big and 2 sub-haploid small nuclei formed. 27. Non-simultaneous behaviour of the two nuclei of the binucleate *p.m.c.*, same as Plate V, Fig. 15. 32. Uninucleate p.m.c. 33. Binucleate *p.m.c.* both the nuclei equal in size.

FIGS. 38–48. × ca 1,800; except Figs. 40 × ca 2,700; FIG. 42 × ca 380; FIG. 43 × ca 900

FIGS. 38–39. Synpollen mother cells showing an attempt at division; compare them with the normal pollen grain. 38 same as Plate IV, Fig. 7.

FIG. 40. Semi-heterotypic division.

FIGS. 41–43. Stages in the fusion of the *p.m.c.*'s; 41 same as Plate IV, Fig. 9.

FIG. 44. Trinucleate *p.m.c.* same as Plate IV, Fig. 8.

FIGS. 45–46. Extrusion of one and three univalents.

,, 47–48. Amitotic division of the T I nuclei.

FIGS. 49–59. FIGS. 49, 50, 52, 53, 54, and 55 × ca 2,700; FIGS. 51, 56, 58 and 59 × ca 3,600; FIG. 57 × ca 1,800

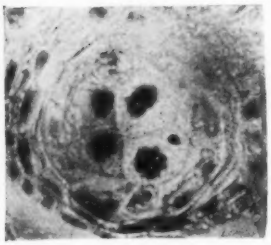
FIGS. 49–51. Bridge configuration due to delayed disjunction.

,, 52–53. Bridge configuration due to the persistence of the interstitial chiasmata.

,, 54–57. Non-disjunction.

FIG. 58. Trivalent lagging on the spindle. Same as Plate V, Fig. 17.

,, 59. Multiple bridge formation due to delayed disjunction and delayed terminalisation; same as Plate V, Fig. 19.



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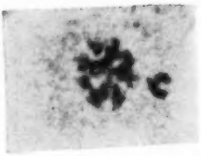

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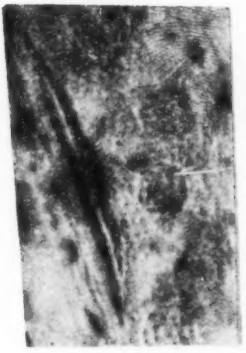

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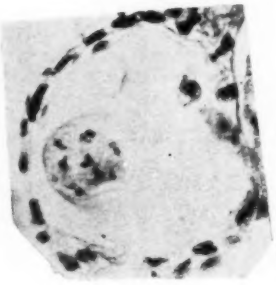

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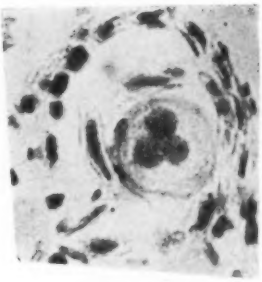

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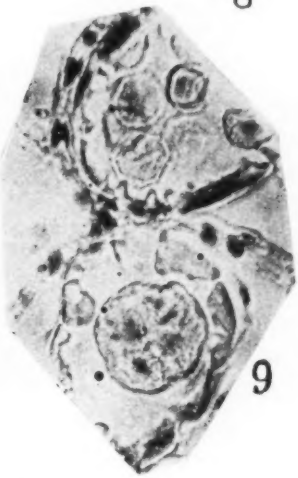

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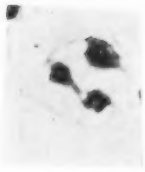

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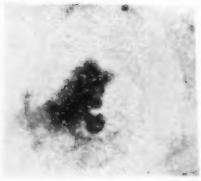

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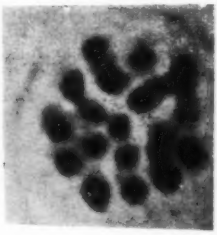

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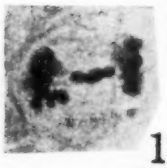

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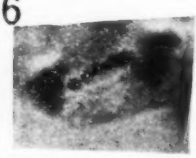

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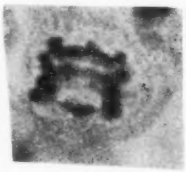

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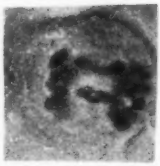

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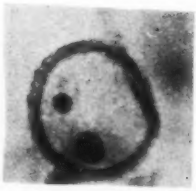

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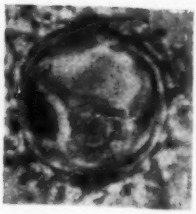

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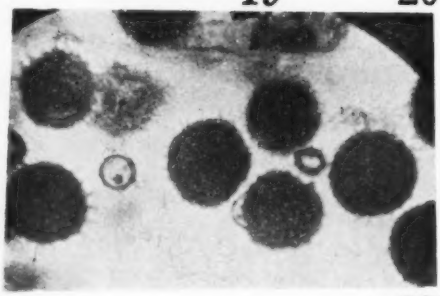

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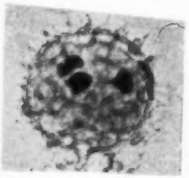

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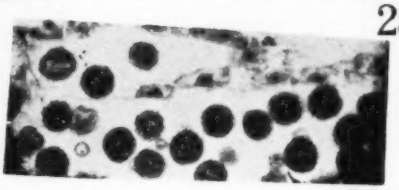

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STUDIES IN THE CAPPARIDACEÆ

VI. Floral Structure in *Crataeva religiosa* Forst., with special reference to the Morphology of the Carpel

By Prof. T. S. Raghavan, M.A., Ph.D. (Lond.), F.L.S.
and
K. R. Venkatasubban, M.Sc.
(*From the Department of Botany, Annamalai University*)

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I. Introduction

In a previous paper (Raghavan, 1939), attention was drawn to some important general structural features of the Capparidaceous flower mainly from the point of view of floral anatomy. In this communication, *Crataeva religiosa*, a genus not dealt with in the previous paper, has been investigated in some detail from an ontogenetic as well as anatomical point of view with the object of throwing some light upon the morphology of the carpel especially. The morphological interpretation of the gynœceum of the Capparidaceæ has been a matter of discussion along with that of the more widely discussed Cruciferæ. In the hope of elucidating further evidence on the subject, this detailed study was undertaken. Though the gynæceum has naturally claimed

the bulk of the paper, the anatomy and developmental history of organs like the gynophore, the septum, etc., have also been described in detail not only because no details of these are available in the literature (so far as we know), but also because it was found that their structure supported the view that has been adopted in this paper, of the nature of the carpel.

The terminology employed in the description and figures is based upon the classical concept of the carpel, that is that carpels form a whorl of only one kind of members, implying a monomorphic as opposed to the polymorphic hypothesis. The placenta is the joint outgrowth from the united carpellary margins. The term commissure is intended to include the region external to the placenta, just at the place of fusion of the carpel margins. In other words, the placenta may be considered as the joint outgrowth from the commissural region.

II. Ontogenetical

The organs of the flower arise in acropetal succession. The origin of the sepal is indicated by a fold in the outer layer. By periclinal divisions of the cells below the fold, the sepals are formed. The petal initials are likewise formed in the second layer of the apex beneath a slight fold in the outermost layer (Pl. VI, Fig. 1). In fact this formation of a slight undulation in the apex always precedes the activity of the cells of the layer below, which results in the formation of the respective organs. It was also observed that the sepals do not originate either from the same level of the axis or simultaneously. Their ontogeny reveals a spirality. The median posterior petal is the first to be formed. After the cells of the petals have been initiated and have grown to some extent by repeated divisions, the apex which was almost conical (Pl. VI, Fig. 2) assumes gradually a broadened form (Pl. VI, Fig. 3). This broadening takes place in such a manner that there is a central rounded protuberance. Histologically this broadening is accomplished by repeated periclinal divisions in the second layer of the apex except at the central dome. In the formation of the stamen primordia the usual folds are formed in the " shoulder " (Newman, 1936) and by cell divisions in the layer beneath the folds, the stamens arise. After all the stamen primordia have been initiated, the central dome shows a fold in the outer layer of cells on one side (Pl. VI, Fig. 4) and the cells below the fold form the carpel primordium by division. It is important to remember two facts, firstly that the two carpels of which the gynæceum of Crataeva is composed, do not arise simultaneously, nor in the same level on the dome. They exhibit a spiral sequence even as the other floral members. Secondly, the initials of the carpels are towards the two sides of the domed apex and never in the centre of the dome. The two carpels arise as infolded structures from the margin of the dome and as

they grow towards one another around the dome the carpellary margins fuse leaving the extreme apex of the dome unused. The full interpretation of this is discussed at the end of the paper. Pl. VI, Fig. 5 shows the two carpel primordia in longitudinal section having grown some length and the swelling represents the unused apex of the dome and the difference in level of the origin of the carpels is also clearly seen. The fusion of the two carpels along the margins is clearly seen in a transverse section at about this stage, and as this has already been demonstrated for Gynandropsis in Pl. XII, Fig. 1 of the previous paper (Raghavan, 1939), that stage is not reproduced here again. Pl. VI, Fig. 6 is a longitudinal section of gynæceum of about the same age.

Some interesting observations were made in connection with the origin of the floral whorls and the members thereof. In the differentiation of the calyx the median posterior sepal is the earliest (Text-fig. 1). This is made out not only by its first separation but also by the vascular strands of the central stele leaving for this organ earliest. Following the ontogeny of the sepals as could be inferred from the differentiation of the members of the calyx whorl, one finds that it follows a clockwise direction in the matter of their release (Text-figs. 1–4). Next to the posterior sepal the right-handed lateral, then the anterior and lastly the left-handed lateral. This implies unmistakably two important things, firstly that the organs do not arise simultaneously and secondly they do not originate from the same level of the floral axis. In other words, the sepals exhibit a clear spiral sequence in their origin. The same is the case with the petals. The members of the corolla are distinctly spiral in their differentiation. The earliest petal is the one between the posterior sepal and the right-handed lateral. And the interesting point is that in their differentiation an anti-clockwise direction is observed. In the matter of the andrœcium the differentiation of the staminal whorls in a spiral manner though quite evident as a whole, their large number made it difficult to follow the differentiation of individual members of the different whorls. It can however be made out that the 28–29 stamens which usually compose the andrœcium, are in three whorls of eight, four and sixteen and in the differentiation of these, one finds that while the first whorl exhibits an anti-clockwise direction its immediate next shows a clockwise direction. This disposition in whorls can, however, be made out by close observation of very young flower buds, especially the release of the vascular supply for the developing stamens. In a slightly older stage all these whorls are jumbled together giving no indication of their arrangement in such definite cycles.

As regards the carpels, we find that the observation made on longitudinal sections was corroborated by transverse sections of very young

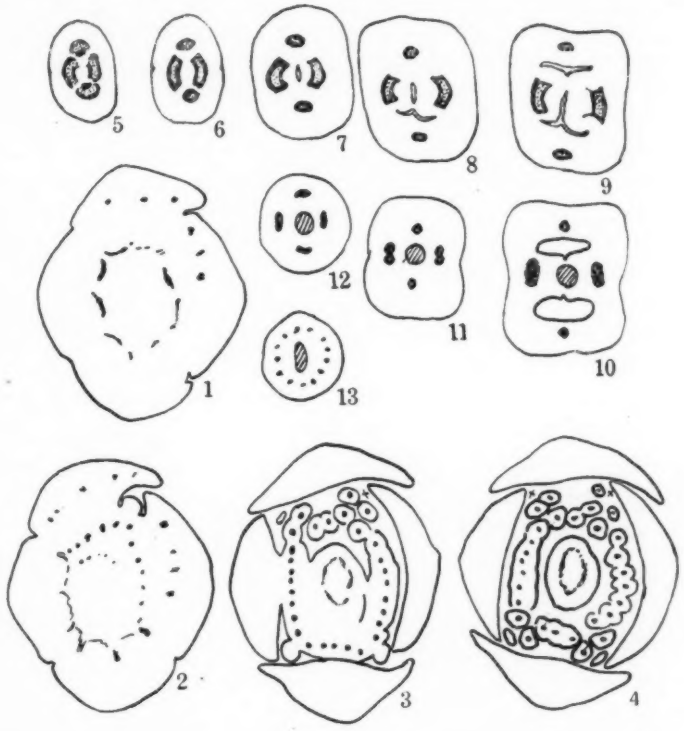
gynæcia. The loculi do not appear simultaneously indicating that the two inrolled carpels by whose marginal fusion the loculi are formed, arise neither from the same level nor simultaneously. Thus the spiral occurrence of the innermost floral whorl, *i.e.*, the carpellary leaves, is also unmistakably evident. The same sequence is observed in the disappearance of these members. The earliest petal to differentiate is the first to disappear; so also with the stamens and the carpels. This also implies that these organs do not arise from the same level of the axis.

III. Anatomical

At the base of the flower bud, there is a ring of vascular bundles. From this the strands for the posterior median sepal are the first to separate (Text-fig. 1). Next the lateral sepal strands are given out (Text-fig. 2). The median sepals are the outer and overlap the lateral (Text-fig. 3). After the petal strands are differentiated the bundles of the stamens separate in a spiral sequence. Most commonly there are twenty-eight stamens for which there are as many bundle supplies. As already stated, in the differentiation of the stamens and their vascular supplies a spirality can be unmistakably traced in respect of each whorl. By tracing the exit of these stamen strands the disposition of the staminal whorls can be inferred. There are eight outermost stamens two on either side of and internal to each petal. Alternating with these pairs are four stamens forming the next inner whorl. Internal to this is a whorl of sixteen.

After the supplies for the stamens have left, there remains the receptacular stele from which the vasculation of the gynæceum originates (Text-fig. 3). Theoretically this residual stele represents the termination of the axis. Arising laterally from this axis are the two carpels which by the fusion of their inrolled margins forms initially a unilocular gynæceum. At the base of the ovary, there is a ring of four prominent vascular strands, two median and two lateral. The loculi make their appearance one by one in a median plane. That is, the carpels are disposed in the antero-posterior plane, a condition which was also recorded for *Euadenia eminens* (Raghavan, 1939). It may be added that generally in this family the carpels are lateral as seen from the drawings of Payer (1857) and Eichler (1875). The lateral bundles are more prominent and bigger than the median. Connecting these are very small minor strands. When the top of the gynæceum is reached we find the lateral bundles showing distinct duality and this is correlated to the spitting of the placental region which delimits the two individual carpels. It is obvious that these two bundles are the marginal strands of the adjacent carpels, which in the lower level of the ovary are in a united condition. The two median bundles represent the midrib bundles of the two carpels. At very

young stages of the ovary when the ovule primordia have not yet been initiated these marginals alone constitute the only bundles at the commissural region. Later on when ovules are being formed one finds a few changes. Firstly, the united marginals assume a concentric configuration



with the phloem almost surrounding the xylem. Secondly, strands passing to ovules make their appearance (Pl. VI, Fig. 7). These may be designated the placental strands. The duality of the marginal strands is a clear indication of the formation of that region by the fusion of the adjacent carpellary margins. One of the reasons of Saunders (1923) for calling this region the 'solid' fertile carpel is that near the top of the receptacle there remain, after the stamen traces have departed, four bundles. These she considered as the four carpel traces. But as we have shown, these are not carpel traces, but represent the receptacular stele which consists of four bundles. The flower is a highly metamorphosed shoot and has a stele made up of discrete bundles which are still further divided by the gaps of the traces proceeding to the floral organs. So the bundles remaining after the departure of the

uppermost stamen traces are not true carpel traces but represent the stele of the receptacle. From these, strands are given off for the innermost floral whorl, the carpels.

IV. Ontogeny of the Gynæceum

By studying the gynæceum at different stages of its development, some interesting observations were made. At a very early stage one is able to identify the duality of the carpellary margins at the placental region and this is very much clearer near the top of the ovary than lower down. It may be added that care was taken not to confuse structural changes occurring in space with those occurring in time. The adjacent margins soon fuse and a clear space in the form of a narrow strip is visible separating the two commissures (Pl. VI, Fig. 8). At this stage of development, the gynæceum is unilocular with two parietal placentas. A little later, the middle portion of each placenta slightly protrudes into a gentle convexity in such a manner that they both fuse in the centre, converting a unilocular into a bilocular ovary (Pl. VI, Fig. 9). A critical examination of this fused portion reveals that there are one or two layers of cambial cells dividing tangentially. The cells have very prominent nuclei with no vacuoles. As the ovary advances in age, a clear partition wall is formed connecting the two placentas (Pl. VI, Fig. 10), obviously due to the activity of the cambial cells referred to above. Thus the ovary is distinctly two-celled till about the stage of fertilization. The ovules are anatropous till after fertilization and the campylotropous curvature is initiated only thereafter. The ovules increase in size greatly and so does the ovary as a whole. This results in the rupture of the septum (Pl. VI, Fig. 7). To our mind this rupture is brought about primarily by a spatial necessity. Near the torn ends of the septum can be seen very distinctly ordinary vacuolated parenchyma cells arranged one above the other in such a manner as to recall to one's mind the secondary cortical cells developed during periderm formation. Obviously these rectangualr cells superimposed in a serial fashion are the products of the activity of the cambial strip mentioned above. And it is also safe to infer that rupture has taken place at the cambial region. From now onwards the ovary (or rather the young fruit) is unilocular right up to the mature stage. In tracing therefore the ontogeny of the ovary, one finds that it is unilocular at a very early stage, bilocular till after fertilization and again attains the unilocular condition during the post-fertilization changes.

V. The Gynophore

(*a*) *Ontogeny*.—The gynophore is an organ of frequent occurrence in the Capparidaceæ. In genera like *Gyanandropsis* and *Cadaba*, there is present

an androgynophore by which not only the gynæceum but also the androeceium, is raised far above the level of the flower. In *Cratæva religiosa*, there occurs a very long gynophore bearing an almost spherical ovary at its tip. Although an organ of such frequent occurrence little is known of its anatomy or of its developmental history relative to that of the ovary it carries. In an ontogenetic study of the floral organs, it would be of interest to find out how and at what stage of the formation of the carpels, the gynophore comes to be formed.

In the earlier stages, it was shown that the carpels arose as inrolled organs from the sides of the floral axis, leaving the extreme apex unused. The carpellary margins fuse forming a unilocular ovary with two parietal placentas. The primordia of the ovules arise from the placental regions and till this stage there is no sign of the gynophore (Pl. VI, Figs. 5 and 6). The subsequent formation of the gynophore is due to the activity of a few meristematic layers at the base of the gynæceum. These cells are arranged in linear rows indicating their having been cut off by repeated tangential divisions of the cells of the meristematic layer. It is by such repeated divisions and subsequent enlargement of these cells that the gynophore attains its length.

(*b*) *Anatomy*.—At the base of the young gynophore is an ellipse of vascular bundles (Text-fig. 5) in conformity with the general configuration of the gynophore at that region. A slight depression is also seen in the middle of each long side of the ellipse. Though the vascular strands are placed so close together as to obscure to some extent their discreteness, it can be made out that four bundles constitute the ellipse. Of these, two lie in the median plane and two along the lateral plane. The terms median and lateral are used relative to that of the floral axis. That is in the same sense in which the outer pair of sepals are the median pair and the other at right angles to the former, the lateral pair. The lateral pair are situated along the long sides of the ellipse and are much longer than the median pair which are disposed along the short sides. These four strands are sheathed on the outside by a sclerenchyma band; so is the case with the inner margin, so that a hollow region of parenchyma is left as a central ring. As one proceeds higher up, the ellipse gradually becomes circular and correlated to this is the fact of the outer sclerenchyma sheath curving inwards at the boundary of each vascular strand in such a manner that soon each bundle comes to be surrounded by the sclerenchyma sheath (Text-fig. 6). The four strands, while closely appressed at a lower level, become separated higher up and at the base of the ovary, the gynophore shows four widely separated

strands, each pair at right angles to one another (Text-fig. 7). The lateral pair still continues to be the bigger. The ovarian cavities make their appearance one after another and from now upwards the unilocular ovary becomes prominent (Text-figs. 8 and 9). It can now be seen that the lateral bundles which were quite prominent right from the beginning of the gynophore; are the fused marginals of the adjacent carpels (Figs. 10 and 11). Further up, at the top of the ovary, a splitting is seen in the placenta demarcating the individual carpels and correlated to this is the division of each united marginal into two, one going to each half placenta on either side. In other words, one strand comes to lie in each margin of the carpel. The transmitting tissue appears still further up (Text-fig. 10), and the duality of the marginal bundles is maintained throughout (Text-figs. 11 and 12) except at the extreme top of the stigma where all the strands lose their identity due to splitting and form together a diffuse vascular cylinder of a number of minor strands (Text-fig. 13).

The close aggregation of the four bundles in a tubular fashion at the base of the gynophore is undoubtedly a result of the mechanical need of the organ which has to withstand a bending strain. And hence it is that one finds that the ring of vascular bundles reinforced both inside and outside by a sheath of sclerenchyma. As the top of the gynophore is reached, the individuality of the bundles becomes gradually clear and no sooner is the top of the ovary reached than the duality of the marginal bundles becomes evident.

It is needless to say that it is these marginals, that when traced downwards to the gynophore appeared as the elongated lateral strands and these by a close approximation with the median which in their turn are the downward continuation of the midrib bundles, formed a compact ring. Ontogenetically it was shown that the gynophore was brought into existence by the meristematic activity of the base of the gynæceum. It is now found that anatomically it conforms in all details to the vasculation of the base of the ovary.

It was said that the carpels originated as two inrolled structures from the margins of the domed apex leaving the extreme tip unused. And in tracing the vasculation of the gynæceum it was found that the base or the lower portion of it exhibited four strands of which the median were the midrib bundles and the lateral represented the united marginals. Their duality became distinct as the top of the ovary was reached. If we imagine the basal portion of the ovary to undergo elongation, however great, naturally the strands originally present at the basal portion of the ovary

also elongate proportionately and hence it is that we find that the vasculation of the gynophore is essentially that of the ovary. But due to mechanical requirements of the organ, the strands have become closely aggregated and reinforced by the occurrence of sclerenchyma sheaths around them. Being a long organ and having to bear a weight at the top in the shape of a heavy ovary, the organ is subjected to the strains of bending to which a normal aerial stem is exposed. And hence we find the bundles in the form of a compact ring sheathed by sclerenchyma. The important thing to remember in connection with the gynophore is that it comes to be formed only after the carpels have been fully formed, more or less in a secondary manner by the meristematic activity of the gynæcial base and as such it possesses the vasculation of the latter though in a slightly modified manner in keeping with the mechanical requirements.

VI. Discussion

(*a*) *The Floral Whorls.*—The nature of the floral organs occurring in *Cratæva religiosa* and the indications they show for the interpretation of the floral parts in general and the carpel in particular will be briefly discussed.

That the flower is a ' determinate branch ' (Eames and Wilson, 1930) is to our mind demonstrated amply by the ontogeny of the floral whorls. Not only do the forms of the various members imply this concept, but the fact that the members of each whorl arise in a spiral, also supports this. One may find something incongruous in using the term spiral, in describing a whorl. Capparadaceæ belongs to the cyclic series of the dicotyledons and as such exception may be taken to the use of the word spiral in the description of the floral members. Though there are various floral whorls, the members of each whorl arise in a spiral sequence. If the ontogeny of the sepals is followed, one finds that seldom do they originate simultaneously; so the petals and the stamens. Even in the matter of the gynæceum it has been shown that the carpels are initiated in a spiral manner. In Pl. VI, Fig. 4 only one of the two carpel primordia is in evidence while the other arises later on and in a slightly different level. This is also indicated by the appearance and disappearance of the loculi in a spiral sequence. Coulter and Chamberlain (1903) doubted whether the members of a whorl ever arose simultaneously. Newman (1928) came to a similar conclusion in respect of the members of each floral whorl in *Doryanthes excelsa*, though according to Engler, the Amaryllidaceæ belong to the cyclic series of monocotyledons. This spiral origin could however be traced only by a critical examination of the ontogeny of the various floral whorls.

The vasculation of the members of the floral whorls fully bears out their foliar nature. Each whorl consists of members arranged in close spirals and to each member pass out as traces the stelar bundles of the condensed floral axis. The vasculation of the latter is that of an ordinary branch or vegetative axis composed of discrete bundles. And just as stelar bundles of the axis leave as traces for the vascular supply of the leaves, so also are gaps formed by traces to the floral organs. These gaps are of course soon bridged and some of these again pass out as traces to the next upper whorl and so on.

(*b*) *The Carpel.*—The carpel needs some special mention, for while the foliar nature of the other floral whorls is more or less generally accepted, the morphology of the carpel has been the subject of wide controversy. McLean Thompson (1929, 1934) for instance, abolishes the carpel altogether. His views on the angiospermic flower and its parts may be summarised as follows: the flower is a sporogenous axis the basal portion of which is sterile while the superficial portion is potentially sporogenous. Bracts, sepals, etc., are products of the sterile base. Microsporangium is an emergence from the lower portion of the sporogenous tissue. Toral growth is commonly dominant over apical growth from an early stage. It may extend beneath the bracteoles and sepals and consequently the maturing axis is cup-shaped when toral growth prevails. An inferior ovary is thus initiated. The emergences which are diverted from spore production mature as stigmatic organs each of which may be a component of a future style. Thus stigmatism is merely a state of tissue. The superior ovary results by the erection of prominent emergences (carpels) from the megasporangium consequent upon the maintenance of apical growth in the axis. On this theory the ovule bearing organ of the Leguminoseæ is a flattened phylloclade. According to him so far as the gynæceum is concerned, the erection of emergences by toral growth known as carpels implies nothing of transmission by descent. All that is obligate by descent is a sporogenous axis which may be moulded according to the nature of its growth. The whole problem to him is one of physiology of the growth of the sporogenous axis.

Barring this a carpellary theory, the other chief views on the morphology of gynæceum grant the existence of such a thing as the carpels and its foliar concept. The classical view of the carpel implies a monomorphic concept. First propounded by Goethe (1790) and regarded by Brown (1840) as generally accepted, the classical theory of the monocarpellary gynæceum is that of a modified leaf (megasporophyll) with the margins fused to form the placenta, the apex of the folded carpellary leaf forming the stigma. This

has been restated and elaborated by Goebel (1905) in his classical organography. The polymorphic concept of the carpel (Saunders, 1923, 1925, 1929) and its fancifulness was discussed in some detail in a previous communication (Raghavan, 1939). So far as it affects the Capparidaceæ, it may be stated that it is said to be composed of carpels of two types, the solid fertile and the sterile valve carpels. Thus it implies that the usual three main functions of the gynæceum are divided between the two types of carpels. The solid fertile concerned with reproduction and reception (being ovule bearing and stigmatic) while the sterile valve has only to form the protective covering of the ovary.

Having these in our minds let us consider what the observations regarding the Capparidaceous gynæceum herein recorded would lead us to conclude. For this we have on hand some amount of anatomical and ontogenetic data. Tracing the developmental history of the gynæceum right from the earlier stage, one finds, that as with the other floral members (sepals, petals and stamens) the carpels also arise in a spiral sequence, never simultaneously, nor from the same level of the axis. Pl. VI, Fig. 4 shows a very early stage of the initiation of the carpels primordia. Only one of the two carpels has been initiated. The other arises later and at a slightly higher level. Similarly at a slightly later stage when the loculi are just being formed by the fusion of the adjacent carpellary leaves, one finds that the cavities appear in a spiral order and not simultaneously; the same sequence is followed in the disappearance of the two loculi. These facts necessarily imply that the carpel must be in the nature of a leaf or a leaf-like organ. If it is morphologically a leaf, though very greatly metamorphosed beyond all recognition, then its origin from the axis must throw some light also. Leaves, whether ordinary or metamorphosed, are always lateral in origin. Never do they terminate the growing apex. In a normal flower, however, one finds the innermost floral whorl, namely the gynæceum, right in the centre in such a position that if one examines the flower even at a very early stage of development, one would unhesitatingly conclude that it has terminated the apex of the floral axis. In other words, in the formation of the carpels, the whole of the apical region including the terminus, has been involved. This can be disproved however by a critical study of the manner of initiation of the carpel primordia from the domed apex. It was stated that after the initiation of each floral whorl—and it must be remembered that the members of each whorl arise in spiral succession—say, petals, the periclinal divisions of the cells extend from the petals to about a third of the distance on the convex upper surface of the axis and this results in the formation of a smooth slope or shoulder from which the central dome stands out prominent. And on this shoulder are initiated the stamen primordia. It is from this what may be

termed the residual axis that the carpels take their origin. It will be seen from an examination of the photographs that one of the two carpel primordia arises from one *side* of the apical dome, while a bit later and at a slightly different level the second carpel primordium originates from the opposite *side* of the domed apex. In a longitudinal section (Pl. VI, Fig. 5) these carpel primordia appear as finger-shaped protuberances. But actually these are in the nature of two incurved semicircular outgrowths from the two opposite margins of the domed axis. Naturally the extreme apex is left unused. And in a longitudinal section we see in the centre of the dome between the two carpel primordia a darkly stained tissue which obviously represents the unused apex of the dome. In a slightly older stage, when the two carpels have grown some distance, there is what may be termed a swelling at the base of one of the carpels. This indeed represents the residual unutilised apex and its position more towards one side rather than right in the middle of the two carpels, is due at once to the non-sumultaneous origin of the two carpel primordia and their initiation at slightly different levels along the margin of the domed axis. Arber (1938) has described the four carpels of very young ovaries of *Papaver argemone* Linn. as surrounding a minute central area of tissue which she calls the vestigeal apex of the floral axis. These observations prove beyond doubt that so far as it pertains to their origin the carpels are definitely lateral and not apical and much like that of any lateral member, a leaf.

The anatomical evidence on hand also supports the foliar conception of the carpel. The flower is a highly condensed shoot of limited growth. The vasculation of the floral axis is that of an ordinary vegetative axis, composed of discrete strands. The vasculation of the various floral members, as has been described already, closely conforms in all essentials to that of a normal leaf. The vasculation of the carpels is essentially that of a lateral appendage, leaf. Each carpel has a dorsal bundle representing the midrib and two ventral bundles representing the marginal strands taking their origin from the receptacular stele. The duality of these marginal bundles does not become clear till the top of the ovary is reached. At lower levels of the ovary, they are single being the united marginals of the adjacent carpels. Higher up, slits between appressed placentas appear, separating the two individual carpels. In correlation to this splitting, the fused marginals divide, each passing to the carpel to which it belongs. One of the arguments for considering the fused carpel margins with their placental outgrowths as the contracted carpels (Dixon, 1935) is that the vascular bundles of the contracted carpel remain unchanged throughout their course. In the present investigation it has been amply shown that the fused marginal bundles do

not remain unchanged throughout their course but each invariably separates into two halves near the top of the ovary, one half passing into the carpellary margin on either side.

The placental strands on whose inverted nature (relative to that of the marginal) Eames and Wilson (1928, 1930) based their interpretation of the Cruciferous and Capparidaceous gynæceum, make their appearance only at a comparatively late stage when the ovules have been formed (Pl. VI, Fig. 7). Itwas shown that at such a stage the united marginals assume a concentric configuration. When a portion of such a concentric strand is detached on the side towards the placenta, it naturally has its xylem turned towards the parent bundle. Thus the inversion of the placental strands can be explained. The reason why the united marginals assume a concentric structure is explained by Arber (1938) on quite simple mechanical grounds. Each placenta is internal to these fused marginals. A collateral bundle cannot supply a vascular system for a structure internal to itself on the same radius. It is a special case of the general problem of how a collateral bundle can give off a branch on its xylem side. In *Cratæva* this problem is solved by the bundle becoming concentric. Therefore much importance cannot be placed upon these inverted placental strands. Thus from the evidence on hand there is no reason to call the commissural region as anything other than the fused carpellary margins. There is no evidence at least so far as the gynæceum of *Cratæva* is concerned to endow this commissural region with the individuality of a distinct type of carpel, the solid.

Since this paper was prepared for the press, we have seen a paper by Kausik (*New Phytologist*, Vol. 37, p. 396) on the morphology of abnormal flowers in some Angiosperms. Some of his observations seem to corroborate the view herein recorded of the morphology of the carpel. In *Allamanda grandiflora* in some abnormal ovaries he has recorded a single terminal vegetative bud between the two carpels (*cf.* his Fig. 3 *e*). This, to our mind, is a clear case of an exaggeration of the growing point which normally is left unused during the formation of the carpels. In another case he has noted two leaf-like carpels. The author himself says (p. 406) that the carpels are the nearest placed to the growing point of the floral shoot and the latest formed *lateral* organ—an observation which has been proved in this paper by detailed ontogenetic and anatomic studies. The author regards these recorded cases of virescence and phyllody as reversions in which there is a reappearance of ancestral characters. In the same breath he says however that " it is not even remotely suggested that the floral parts are in any sense metamorphosed foliar appendages ". What else are these cases of reversions to, it is hard to

understand, unless it be that these are considered as organs *sui generis*, of which there is however no mention.

There are also some interesting observations in respect of what is called " gynophore " which occurred as an abnormality in *Allamanda*. Its anatomy according to the author would appear to conform to the vasculation of the floral axis, composed of a ring of vascular bundles. In that case these would be true stelar bundles from which the carpellary strands must be given out. Theoretically these stelar bundles would end somewhere, denoting the termination of the axis. Thus according to the author the gynophore originates as an elongation of the floral axis. This is only an inference that we are led to, as there is no detailed account of the ontogeny of the gynophore-like organ. We would only point out that in the case of *Cratæva* where the gynophore is an organ of normal occurrence, it is formed by the elongation of the basal portion of the ovary; its vasculation is essentially that of the gynæceum and not of the axis. How far the structure of a normal gynophore and that of one that comes to be present only as an abnormality, differ from one another in this important detail, can be confirmed and generalised only after further critical comparative examination of gynophores in other genera of the Capparidaceæ and those occurring in a state of atavism.

(*c*) *The Septum.*—A word may now be said of the septum that is a feature of constant occurrence in the ovary till after fertilization. It consists of a partition stretched between the commissures. At low levels of the ovary the septum is thicker than at a higher level. Ontogenetically at a very young stage the two commissures are just free so that the ovary is unilocular. Later the central portion of the commissures touch one another, a narrow strip of meristem is formed by whose repeated tangential divisions the septum is brought into existence. Rupture takes place presumably at the cambial region and the bilocular ovary becomes uni-locular once again after fertilization. The occurrence of what is termed a spurious septum is sometimes described in connection with the Capparadaceæ. No detailed account of the method of its origin or its duration in the different genera of the family is available. It was therefore considered desirable to trace its origin and fate. The septum lasts till after the fertilization of the ovules. In the related genus *Cadaba indica* the septum is a permanent feature. It is present for a brief space of time in genera like *Cleome* and *Gynandropsis*. It is therefore inferred that the septum formation in this family may throw light upon the formation and the nature of the septum in the Cruciferæ, where it is a feature of constant occurrence and which has been the subject of discussion at the hands of morphologists. The morphology of the Cruciferous

septum to which terms like dissepiment, false partition, replum, etc., are variously applied, has been differently interpreted.

de Candollé (1821) believed that the two carpels were fused together as in a normal syncarpous ovary with axile placentation; the septum then would be made up of the ventrals or the margins of the two carpels. Chodat and Lendner believe the septum to be the receptacle prolonged and flattened. Payer (1857) and others have held that the septum is a placental outgrowth. Eames and Wilson (1928) hold that the septum is carpellary in nature. It must be remembered that according to them the commissure of the classical concept of the gynæceum represents the solid carpel, in which the loculus has wholly disappeared and the usual dorsal and ventral strands are present in close apposition. On this basis, the part lying inward of the inverted ventral bundles must represent the narrow strip of tissue on the ventral margin of the folded carpel. This strip in this case is said to be much extended and forms a broad wing on the reduced carpel. The wings of the two carpels meet in the centre and the septum is formed.

Applying these views to the Capparidaceous septum, which to our mind is very similar to the Cruciferous ceptum, we find that on the evidence of ontogeny, the septum is unmistakably the product of activity of the cambial strip which is formed at the place of the union of the two commissural margins at a very early developmental stage of the gynæceum. Definitely it is a lateral (relative to the floral axis) placental outgrowth of a secondary nature. A critical examination reveals that not the entire fused margin of the placenta forms the meristematic strip. It is formed in the middle. Each carpel is an inrolled leaf so that the tips are free to bear the ovules. True it is that it is the fused product of the adjacent carpellary margins that constitutes the placenta. But in this case the central portion of the placenta takes part in what may be termed the secondary origin of the septum while the ends representing the tips of the inrolled carpels are free to bear the ovules. This can best be seen in Pl. VI, Fig. 7, where the carpellary tips bearing the ovules are seen quite distinct from the central portion in which alone are seen the remnants of the septum.

de Candollé's (1821) interpretation of the septum would involve an axile placentation for the cruciferous and Capparidaceous gynæceum. That this is not so is made clear by a study of the ontogeny of the gynæceum, whose placentation is unmistakably parietal at least in the initial stages. Chodat and Lendner's axillary concept of the septum, would mean that all the axis is prolonged to the top of the ovary. For this, there is neither ontogenetic nor anatomical evidence. There is no stele nor any evidence of a vascular

tissue even of a vestigeal nature in the septum and this should be present if it were axial in nature. Eames and Wilson's (1928) interpretation of the septum as being carpellary, is based on the assumption that the placentas represent the contracted solid carpels. There is no evidence, however, at least so far as Capparidaceæ is concerned to look upon the placenta as anything other than the fused margins of the adjacent carpels. According to them the presence in the septum of the inverted bundles is sufficient proof that the septum is not a mere placental outgrowth, in which there is usually no fundamental vascular system.

In *Cratæva*, no bundles, inverted or otherwise were found either in the septum or even in the placental region. Another argument of theirs in favour of the carpellary nature, is that the outer cell layers of the septum are epidermis like and in some cases provided with stomata. So far as *Cratæva* is concerned, stomata were found only in the abaxial side of the carpellary leaf. None were found either in the adaxial surface or in the funiculus or the septum. Nothing in this paper is said of the origin of the ovule, whether from the abaxial surface of the carpellary margin or from the adaxial. It is therefore with hesitation that we venture to offer an explanation for the presence of stomata in the partition. Stomata are of frequent occurrence on the abaxial surface of the carpel. Rarely they occur also on the adaxial (the side lining the ovarian cavities). If in the species of Eames and Wilson stomata occurred only on the abaxial surface, could it not be that the septum represents an outgrowth of the abaxial margin? This would imply that the ovules arise from the abaxial margin of the carpellary leaf. For such an origin of the ovules there is evidence. Newman (1928) has proved the abaxial origin for the ovules of *Doryanthes excelsa* and also *Acacia Baileyana* (1933). Goebel (1905) also states that the occurrence of ovules on the undersurface of the carpels is not rare. On the other hand there is the possibility of stomata occurring on both the surfaces of the carpel. Orr (1921) for instance has noted the occurrence of stomata in the inner ovaraian wall of *Cleome spinosa* and *Isomeris arborea*. If that is so, this assumption of the abaxial outgrowth is not necessary, for the septum may then be regarded as an outgrowth of the abaxial margin of the carpellary leaves.

Thus from the present investigation the septum is definitely a placental outgrowth of a secondary nature. There is no evidence to endow it with a distinct carpellary affinity. It is, however, carpellary to the extent that the placenta, of which this is an outgrowth, is undoubtedly an important portion of the carpels.

VII. Summary

Floral ontogeny and anatomy in *Crataeva religiosa* Forst., have been investigated in some detail and on the basis of the following observations, some conclusions drawn on the nature of the floral organs, especially the carpel.

The floral organs arise in acropetal succession and the members of each floral whorl exhibit a distinct spirality in their origin. The two carpels are lateral in origin and the terminus (residual apex) is always left unused. They do not arise either simultaneously or from the same level indicating a spirality in their origin.

The vasculation of the floral organs is found to be essentially that of a lateral appendage. The floral axis has a stele of discrete bundles which are further divided by the gaps of the traces proceeding to the floral organs. The bundles remaining after the departure of the uppermost stamen traces are, on this basis, interpreted as the residual stele of the receptacle (which theoretically represents the termination of the axis) and not as carpel traces direct.

The gynæceum is composed of two carpels disposed in the antero-posterior plane, with the parietal placentas made up of the fusion of the carpellary margins. The duality of the marginal strands is also recorded. At first unilocular, a septum is soon developed by cambial activity and this bilocular condition of the gynæceum persists till fertilization. The ultimate unilocular condition is reached by poste-fertilization rupture of the septum.

The origin and development of the gynophore are described in detail. The formation of the gynophore is the result of the activity of a few meristematic layers at the base of the young gynæceum. The anatomy of the gynophore conforms to that of the gynæcial base. The median bundles are the downward continuation of the carpellary dorsal strands, while the laterals which are more prominent, are the fused ventral strands which at ovary region exhibit their duality clearly, especially towards the top. In the light of these observations, the foliar concept of the floral organs (including the carpels) is upheld, and there is no evidence at any rate in this genus to consider what has been found to be the commissures, as the solid carpels. The inverted placental strands which are used as a strong argument in favour of this concept, are found to appear comparatively late. Their inverted nature is merely a case of the general problem of how a collateral bundle can give off a branch on its xylem side. Here this is solved by the fused marginals assuming a concentric configuration and when a portion of it is

detached to supply the ovules, the separated placental strand has naturally its xylem turned towards the parent bundle.

The various views on the nature of the Cruciferous and Capparidacious septum are discussed in the light of the present findings and the conclusion is reached that it is a placental outgrowth of a secondary nature.

VIII. Acknowledgment

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EXPLANATION OF PLATE FIGURES

PLATE VI

All the figures are photomicrographs (ca × 75. Fig. 10 × ca 20).

FIG 1. L. S. of young flower bud. Note the median posterior sepal has arisen earliest; origin of petal primordium from the hypodermal cell in the 'fold'.

FIG. 2. Slightly later. After the petal primordium has been initiated the apex is conical.

FIG. 3. The conical apex has broadend; the stamen primordia are being initiated.

FIG. 4. L. S. The initiation of one of the carpel primordia from the residual apex.

FIG. 5. L. S. Later stage. Both the carpels have been initiated. Note the swelling towards base representing the vestiges of the residual apex.

FIG. 6. L. S. still later stage. Only one of the two carpellary chambers has appeared.

FIG. 7. The placental region of a mature ovary in T. S. The septum has ruptured the remains of which may be seen to be composed of serially arranged rows of cells. Note also the carpellary ends curved away and quite distinct from the central region.

FIG. 8. T. S. of young ovary; unilocular condition.

FIG. 9. T. S. of slightly older stage; beginning of the bilocular condition.

FIG. 10. T. S. of mature ovary (central portion alone shown) showing the septum.

TEXT-FIGS. 1–4 (× ca 80)

Serial T. S. of young flower bud at sucessively higher levels.

TEXT-FIG. 1. The separation of the posterior median sepal and next the right-handed lateral. Note the vascular supplies of these have already left the central stele.

TEXT-FIG. 2. The origin of the first petal between the median posterior and the right-handed lateral. Note the separation of the staminal strands in a spiral manner.

TEXT-FIG. 3. The differentiation of the sepals in a clock-wise direction. The first petal to appear (*i.e.*, the lowest) has disappeared at this level, and the next towards the left has almost disappeared. Thus in the differentiation of he petals an anti-clock-wise spiral direction is followed.

TEXT-FIG. 4. T. S. of the base of the ovary. The posterior petals have disappeared. Note the arrangement of the stamens (the filaments) in whorls and each whorl being a spiral).

TEXT-FIGS. 5–13 (× ca 80)

TEXT-FIGS. 5– 8. T. S. from gynophore to base of ovary taken at successively higher levels. Description in text.

TEXT-FIGS. 9-13. Serial T. S. from base of ovary to sigma at different levels. Note at the top of the ovary the transmitting tissue (cross-hatched). Still further up (Fig. 11) the duality of the marginal strands. At the stigma there is a diffuse ring of small strands surrounding the transmitting tissue.

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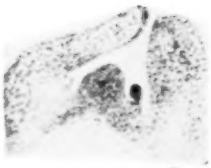

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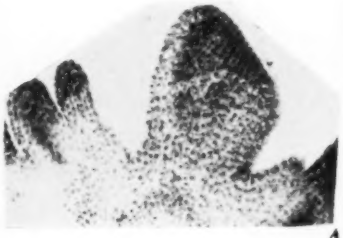

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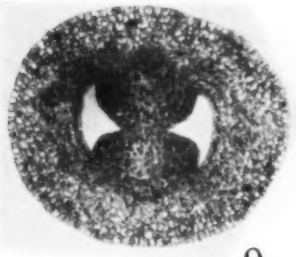

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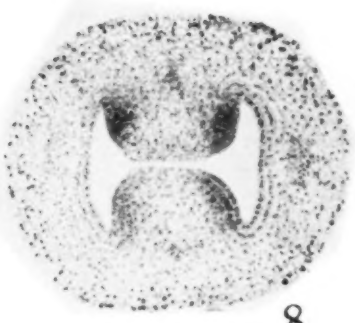

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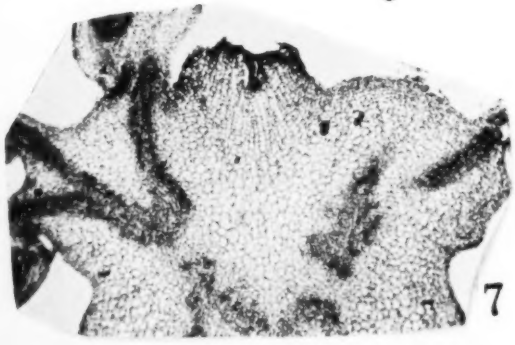

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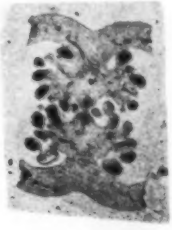

10

THE CHEMISTRY OF GARLIC (*ALLIUM SATIVUM* L.)

Part III. The Reserve Polysaccharides

By C. P. Anantakrishnan and P. R. Venkataraman
(*Department of Biochemistry, Indian Institute of Science, Bangalore*)

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Carbohydrates are generally stored as polymerised polysaccharides in various parts of the plant to be subsequently mobilised to meet any metabolic process that may arise. The storage of carbohydrate in this comparatively insoluble state, *e.g.*, as starch secures its safe retention. The crystalloidal carbohydrates may pass into the soluble, but colloidal and non-diffusible state, like inulin, which is the polymer of fructose.

Fructose seems to be a rather common unit in soluble polysaccharides. Johansson (1899) found several inulin-like carbohydrates in the Gramina. Parkin (1899) found, what he called inulin, fructose-yielding polysaccharides in the bulbs and tubers of several mono-cotyledons, sometimes together with starch. Mono-saccharides other than fructose also build up soluble polysaccharides. Glucose, mannose and galactose are all found as constituents of polymeric soluble carbohydrates.

It is quite possible that the reserve polysaccharides may act as emollients provided they are not attacked in the alimentary canal.

Experimental

1. *Preliminary analysis*

The various forms of carbohydrate present in Garlic are determined by the standard methods.

In this investigation, sugar determinations were carried out by following the conditions of Munson-Walker (1906) for reducing, using Bertrand's method (1906) of determining the copper, by dissolving the cuprous oxide in ferric alum and titrating the reduced iron by a standard potassium permanganate solution. Reducing sugars, sucrose and starch (*Taka-diastase*)

were determined by the A.O.A.C. methods. Dextrins were determined by the method of Totingham *et al.* (1935). Table I presents the results of these experiments.

TABLE I

Expressed as percentage of the fresh material

Reducing Sugars . .	0·14
Sucrose	3–79
Starch	8–22
Dextrin	7–69

2. *Examination of the soluble polysaccharide*: *Preparation of the soluble polysaccharide*

250 g. of garlic was crushed to a fine paste in an end-runner. The paste was suspended in water (1·5 litre), stirred vigorously, and kept at 80° for 24 hours. The hot mixture was filtered through a cloth, and the filtrate was poured into three volumes of alcohol, and the precipitate allowed to settle by standing overnight. The supernatant layer was syphoned off and the carbohydrate was collected on the centrifuge, washed with absolute alcohol, dry ether and was finally dried in vacuum. The yield was 8 g. Nitrogen, phosphorus and ash were determined on the specimen. The results are N, 1·02, P, 1·10 and Ash, 0·6 per cent.

Purification.—The carbohydrate was brought into solution in water and made finally alkaline with sodium hydroxide and reprecipitated by pouring into alcohol. After two such purifications, the polysaccharide still analysed for nitrogen, phosphorus and ash as 1·00, 0·98 and 0·48 per cent. respectively. No further purification was attempted.

It is worthwhile to point out here that polysaccharide containing nitrogen as high as 4·2 per cent. (Black, 1931) from the pollen of Giant weed has been prepared. Similarly, Gough (1932) isolated a polysaccharide containing about a per cent. of nitrogen from Timothy grass pollen.

Properties.—

(1) The carbohydrate swells with water.

(2) In 2 per cent. sodium hydroxide it had $(\alpha)^{25}_{5461} = -41°$.

(3) It is not easily soluble in water, but dissolves readily on the addition of alkali.

Reactions.—

(1) The polysaccharide gave no colouration with a solution of iodine.

(2) It gave Molish's test with sulphuric acid and alcoholic β-naphthol.

(3) Fehling's solution was not reduced in the cold but was reduced readily if the carbohydrate was warmed previously with hydrochloric acid.

(4) Warming with concentrated hydrochloric acid and either orcinol or phloroglucinol did not produce any characteristic colour for pentoses.

(5) Methyl pentose are absent as the Rosenthaler's reaction (1909) is negative.

(6) The polysaccharide failed to produce any characteristic colour for glycuronic acid with naphtho-resorcin (Neuberg and Kobel, 1931).

(7) It gave positive reaction for keto hexoses according to Selivanhoff (1887) using resorcinol and hydrochloric acid.

Hydrolysis of the Polysaccharide.—0·5 g. of the carbohydrate was hydrolysed with 50 ml. of one per cent. sulphuric acid for 3 hours on a boiling water-bath. After hydrolysis it gave a rotation of + 9° (l = 1 dm.). After neutralising the hydrolysate, reducing sugars were determined. This corresponds to 0·47 g. of glucose.

The reactions of the hydrolysate.—It gave no reactions for pentoses, methyl pentoses, glycuronic acid. But the test for keto hexoses was very strong.

Preparation of a barium salt from the hydrolysate.—Two grams of the polysaccharide were hydrolysed with 200 ml. of one per cent. sulphuric acid for 3 hours. The hydrolysate was neutralised with barium carbonate and filtered. The precipitate was washed with warm water and the filtrate concentrate to a thick syrup. The syrup was dissolved in 20 ml. of water and diluted with 200 ml. of methyl alcohol. The precipitate after standing for an hour was collected on the centrifuge and washed with methyl alcohol and dried *in vacuo*. It weighed 330 mg. The barium content was determined by the titrimetric method of King (1932) using 45 mg. of the barium salt. The barium content was found to be 26·8 per cent. It did not reduce Fehling's solution even on warming.

Identification of the products of hydrolysis.—The methyl alcoholic centrifugate after precipitation of the barium salt was concentrated *in vacuo* to

a syrup. The residue was again dissolved in water and evaporated to a syrup. This process was repeated twice over. Finally the syrup was dissolved in 20 ml. of water. More of the carbohydrate was hydrolysed and worked up in this way for examination.

The sugar solution gave rise to an ozazone m.p. 205°, identical with that of the glucose ozazone. Since glucose ozazone could arise from glucose, mannose, galactose and fructose, these units were tested in the hydrolysate. Glucose and galactose are absent because the hydrolysate failed to reduce the Nylander's reagent; oxidation of the hydrolysate or the solid polysaccharide with nitric acid resulted only in the formation of oxalic acid and no mucic acid could be detected. The hydrolysate in cold gave a phenyl hydrozone, m.p. 203–4° (mixed m.p. with an authentic specimen was not depressed). The filtrate after the collection of mannose at the pump, was shaken with benzaldehyde and refiltered. The final filtrate gave the characteristic red colour for keto hexoses with resorcinol and 12 per cent. hydrochloric acid, and the orange red precipitate with thio-barbutyric acid in 12 per cent. hydrochloric acid (Plaisance, 1916). The presence of mannose was confirmed by the preparation of the anilide, m.p. 180° (decomp.) from the hydrolysate (Irvine, 1910). The fructose was identified in the hydrolysate as the *as*-methyl phenyl ozazone, m.p. 156°, confirmed by a mixed melting point with an authentic specimen.

The above series of experiments establish that in addition to starch the reserve polysaccharide of garlic is composed of fructose, mannose and a non-reducing acid. Phosphorus and nitrogen were also found in the preparation. The ash from the polysaccharide gave positive tests for calcium.

Summary

1. The various forms of carbohydrate in the bulb of Garlic have been determined.

2. In addition to starch, the reserve polysaccharide is made up of mannose, fructose and a non-reducing acid.

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ON A NEW *MYXOSPORIDIAN HENNEGUYA OTOLITHI* N. SP. A TISSUE PARASITE FROM THE BULBUS ARTERIOSUS OF TWO SPECIES OF FISH OF THE GENUS *OTOLITHUS**

By P. N. Ganapati, M.A., M.Sc.

(From the University Zoological Laboratory, Madras)

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Introduction

The study of Myxosporidia, primarily a group of Sporozoan parasites in fish, has received considerable attention within recent years and much work in this direction has been done in different parts of the world, especially in North America and to some extent in Japan. Our knowledge about the

* Formed part of thesis for the M.Sc., degree of the Madras University.

Indian Myxosporidia is, however, very meagre, the only contributions on the subject being those of Bosanquet (1910), Southwell (1915), Southwell and Prashad (1918), Ray (1933), Ganapati (1936), and Chakravarty (1939).

The present investigation was taken up with a view to make a detailed study of the life-history, incidence of infection and host-parasite relations of a myxosporidian found as a tissue parasite in the heart of two common species of a local edible fish *Otolithus ruber* (Bl. Schn.) and *O. maculatus* (Kuhl and Hass.). The work was carried out during the years 1935–1938, in the University Zoological Research Laboratory under the direction of Professor R. Gopala Aiyar. I wish to express here my grateful thanks to him for his very helpful criticisms and constant encouragement. I am indebted to the University of Madras for the award of a research studentship which enabled me to carry out the work.

General Observations

There are no external indications to show infection, which is evident only on opening the animal and examining the heart. The parasite forms whitish opaque cysts in the wall of the bulbus arteriosus. The infected organ often presents a corroded appearance owing to the large number of cysts which are covered over by dark pigment granules (Photomicrograph 1). When the infection is localised the contour of the bulbus is considerably changed.

Myxosporidia though found in various tissues and organs have seldom been reported from the vascular system. The only previous record seems to be that by Keysselitz (1908) of *Myxobolus cordis* from the ventricle of *Barbus fluviatilis*. The present form belongs to an undescribed species of *Henneguya*, and is the first instance where the genus has been known to attack the vascular system. This fact together with other major characteristics of spore structure seem to me of sufficient importance to describe it as a new species of the genus for which the name *Henneguya otolithi* is proposed.

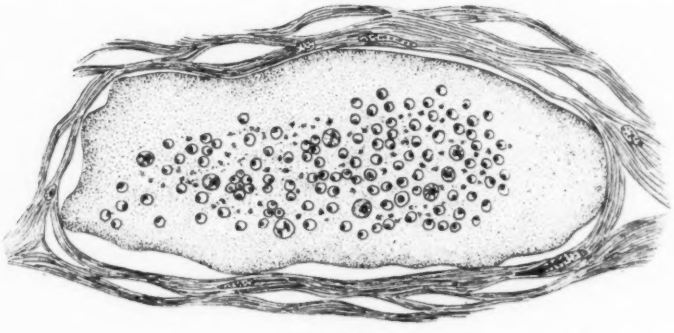
A large number of fish have been examined during the course of three years. *Otolithus ruber* is the commoner of the two species and *O. maculatus* though comparatively rare is equally susceptible to infection by this myxosporidian. Almost 75 to 80 percent. of the young and half-grown fish show infection in different degrees. Very young and full-grown fish are generally free from infecton. It is therefore believed that the host, after passing through a certain stage in its life-time acquires immunity to the attack of the parasite. When infection is not heavy the fish appears to recover. It is not possible to say whether such fish establish immunity to further attacks.

Material and Methods

The fish used in this investigation were bought from local fishermen as soon as catches were brought to the shore. The Laboratory being situated very near the sea-shore, it was possible to examine the fish almost in the fresh condition. The infected organ was at once fixed for section cutting. Almost all the observations regarding the earlier stages and spore formation are based on sectioned material. Several fixatives like Bouin's, Zenker's, Schaudinn's, Flemming's and Worcester's fluid were tried. The last named fixative gave uniformly good results. Sections were cut 6 to 8 microns thick and ordinarily stained in Heidenhain's iron-alum hæmatoxylin followed by eosin or orange G. Ehrlich's hæmatoxylin counterstained by Van Gieson, Dobell's method of Mann's stain and Mallory's triple stain were also tried with success. A dilute solution of potassium hydroxide brings about filament extrusion in about 10 to 15 seconds. Examination of all the internal organs was carried out to study the distribution of the parasite within the host.

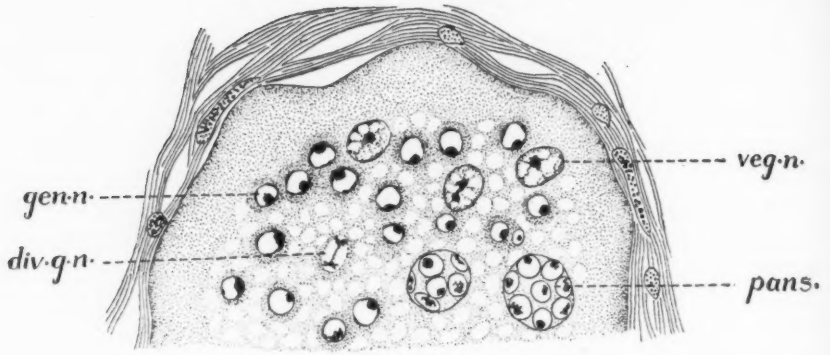
The Trophozoite

The trophozoites or vegetative forms are rounded, ovoidal or irregular. In the earlier stages a definite cyst wall formed of the host's connective tissue is not present and the parasite lies in between the muscle fibres. The body protoplasm is differentiated into an outer thickly granulated ectoplasm and



TEXT-FIG. I

a coarsely reticulated endoplasm (Text-Fig. I). In young trophozoites the endoplasm contains a few nuclei which are of two kinds—the generative and the vegetative. The vegetative nuclei are few in number and measure 3 to 4 microns in diameter. They are spherical to ovoidal in outline with a slightly ex-centric karyosome (Text-Fig. II). The vegetative nuclei are



TEXT-FIG. II

seen only in young trophozoites where spore formation has not commenced. The generative nuclei measuring 1·5 to 2 microns in diameter are much larger in number though smaller in size than the vegetative nuclei. The generative nucleus is rounded with a comparatively large karyosome often applied against a clear and well-defined nuclear membrane. The chromatin in both kinds of nuclei seems to be concentrated in the karyosome and very little of it is distributed in the nuclear sap. A thin layer of cytoplasm staining more vividly than the surrounding area, is present around each generative nucleus, and this feature together with its size and structure distinguishes it from the vegetative nucleus.

The differentiation of the nuclei in Myxosporidia into the two kinds, vegetative and generative, has been observed in all species that have been investigated in detail. Keysselitz (1908) differentiates the two kinds in *Myxobolus pfeifferi* and states that the division of the vegetative nucleus and the formation of generative nuclei are limited to a period in the early trophic life of the myxosporidian. Davis (1923) holds that the vegetative nucleus does not give rise to the generative nucleus after it has differentiated as such. The fact that the vegetative nuclei are present in the early stages of the trophozoite and that they are comparatively rare in older parasites seem to suggest that they give rise to the generative nuclei.

As the parasite grows, nuclear multiplication proceeds at a rapid pace, and the endoplasm in older trophozoites is filled with innumerable generative nuclei. The generative nucleus divides by a form of mitosis. The extremely small size of the nucleus renders it difficult to follow the details of the division. No asters or centrosomes have been made out at any stage of division. The older vegetative forms are more regular and a cyst wall composed of several

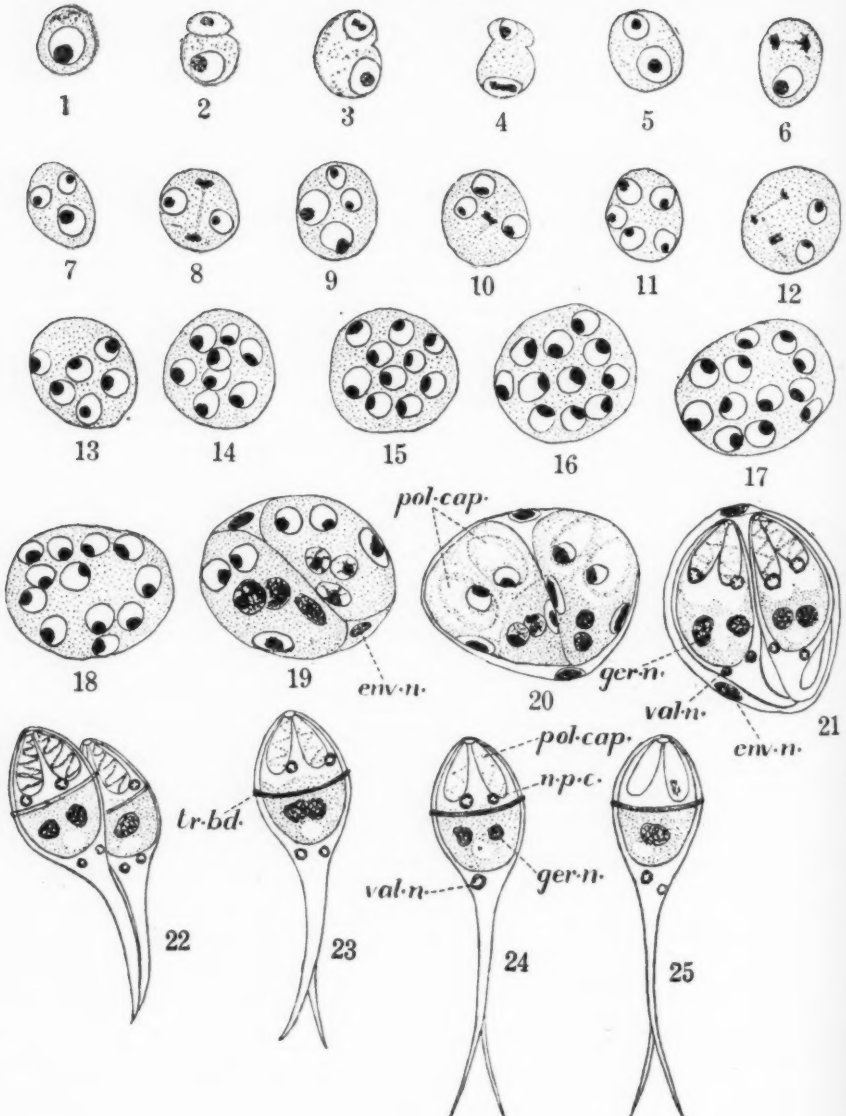
layers of the host's connective tissue is present round each parasite. Such a cyst examined in the fresh condition shows a number of developing pansporoblasts together with some refractile yellowish bodies and a few globules of fat.

Vegetative multiplication by plasmotomy appears to take place in the early stages of the trophozoite. Large numbers of young vegetative forms at the same stage of development have often been seen lying close together (Photomicrograph 2). It is difficult to imagine these forms could have originated by independent infection by different amoebulæ. Some of the pictures presented by these trophozoites show evidences of multiplication by plasmotomy. The occurrence of a multiplicative process of reproduction amongst tissue infecting Myxosporidia has been observed in *Myxobolus musculi* (Hahn, 1913), *M. pfeifferi*, Keysselitz, (1908), *Lentospora ovalis* and *Unicapsula muscularis* (Davis, 1923 and 1924). This process of vegetative multiplication is common in Myxosporidia living in organ cavities.

Sporulation

There is considerable diversity of opinion with regard to the origin of the pansporoblast or sporoblast in those forms where it gives rise to a single spore. Much of the controversy centres round the question of the presence or absence of a sexual process in its formation. Naville (1930) gives an excellent review of the previous literature on the subject and by his independent researches on five species of Myxosporidia comes to the conclusion that in all these forms anisogamous gametes are formed preceded by a reductional phenomena of the chromosomes. The earlier stages of the parasite, according to this author, are diploid, while the gametes are haploid. The pansporoblast originates by the copulation of two anisogamous gametes not necessarily accompanied by nuclear fusion. When nuclear fusion also takes place, the zygote nucleus undergoes a reduction division in one of the subsequent divisions in the pansporoblast thereby again becoming haploid. The copulation of the two germ nuclei in the sporoplasm, either before or immediately after the germination of the spore, is said to restore the diploid number characteristic of the earlier stages of the parasite.

The gametogenesis and their chromosomic constitution have not been worked out in the present form. It has, however, been found that the pansporoblast originates by the copulation of a large and a small cell. These two presumably represent the macrogamete and the microgamete (Text-Fig. III, 1–2). In the two-celled stage a constriction, and in those cases where the protoplasmic fusion is incomplete, the faint outline of a cell wall is visible



TEXT-FIG. III

between the two nuclei (Text-Fig. III, 2–4). The two nuclei do not appear to fuse but proceed to divide independently. Either the smaller or the larger

nucleus may be the first to divide, so that three nucleated stages are common. In the succeeding divisions of the nuclei outlines of cell walls are not visible and pansporoblasts containing up to fourteen nuclei are met with. Two of these nuclei which are smaller than the others occupy a parietal position and they form the nuclei of the enveloping membrane when it is formed (Text-Fig. III, 19).

The different parts of the spore are constituted from the cells of the pansporoblast esssentially as described by previous writers. The twelve nuclei separate into two groups, each of six cells, and a split appears in the cytoplasm separating the two groups (Text-Fig. III, 19–21). The envelope cells as already said occupy parietal positions. Each of the six cells is distributed relative to its position in the mature spore. The two anterior cells enlarge and gradually a vacuole is developed inside them. These two form the polar capsules (Text-Fig. III, 20). The spore valves are formed by the two parietal cells, while the protoplasm of the two remaining cells fuse and form the sporoplasm. In a few spores there was only a single germ nucleus formed by the fusion of the two originally separate germ nuclei. This is clearly a case of autogamy the significance of which is discussed in a later part of the paper. When the spores are fully formed, the enveloping membrane disintegrates and liberate the spores which lie free in the endoplasm of the parasite. The ectoplasm has meanwhile considerably shrunk and it is possible it is made use of by the developing pansporoblasts. When spore formation is complete the cyst is practically filled with spores and a few darkly staining bodies (Photomicrograph 4). Davis observed similar bodies in *Lentospora* and calls them " chromatoid bodies ". Kudo (1926) noted these bodies in *Myxosoma catostomi* and is inclined to take them as different stages of degenerating nuclei. The staining reaction of these bodies evidently suggests their probable nuclear origin but beyond this it not possible to indicate their true nature.

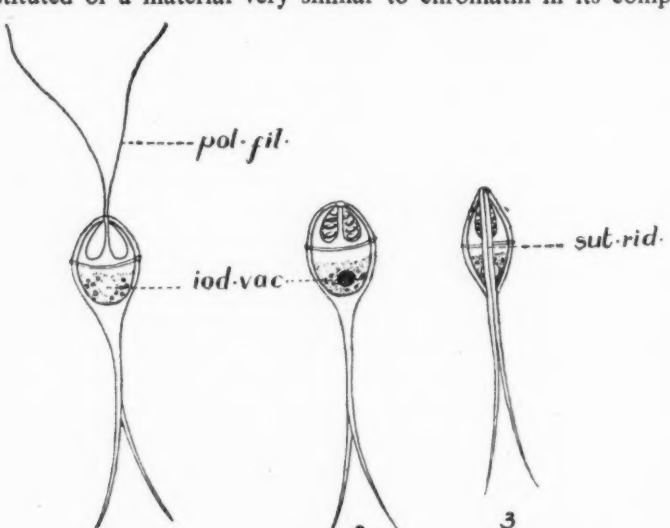
Description of Spore

The spores are oval in front view with rounded anterior ends. The two shell-valves are prolonged posteriorly into finely tapering tails. In profile, the spores are spindle-shaped. A thick, straight and simple sutural ridge is present. The shell is unstriated, but a characteristic thickening is present running transversely about the middle of the main part of the spore. The presence of this thickening or band enables the present species to be distinguished from other described species of *Henneguya*. Two pyriform polar capsules occupy the anterior third of the spore cavity and they are

convergent with a common opening. A spirally coiled polar filament showing five to six turns is visible in each of the polar capsules. The sporoplasm is finely granular and contains an iodinophilous vacuole.

The dimensions based on measurements of fresh spores are as follows: length 10 to 12 microns; breadth 6 to 8·5 microns; thickness 4 to 5 microns; length of polar capsules 3 to 4 microns; breadth of polar capsule 2 to 2·5 microns; length of tail 35 to 40 microns.

The nuclei of the polar capsules and the spore valves persist for a considerable time after the spores are fully developed (Text-Fig. III, 22–25). The nuclei of the capsules are in the form of chromatin rings with a few beads of chromatin distributed along the nuclear membrane. The valve nuclei are much shrunk and lie at the root of the bifurcated tail. Spores in different stages of development show an interesting gradation of basophilic affinity as shown by their reaction to nuclear strains. With iron-alum hæmatoxylin, spores having the full quota of six nuclei, have their spore valves and appendages unstained, while their nuclei are stained deep. As the spores mature the basophil constituents seem to increase and ripe spores are stained intensely and remain so even after being strongly counterstained with acid dyes like eosin. The nuclei of the valves and appendage can scarcely be made out and presumably they degenerate when the spores ripen. The basophilic affinity indicates that the shell and appendage of the spores are constituted of a material very similar to chromatin in its composition.



TEXT-FIG. IV

By treating fresh spores with a dilute solution of caustic potash, the polar filaments may be made to extrude in about ten to fifteen seconds. The extrusion is accompanied by a sort of recoiling movement of the spore as a whole and it would appear as though a release of pressure has been effected inside the spore cavity. The sporoplasm in fresh spores shows a clear hyaline rounded area in the centre which goes dark brown when the spores are treated with Lugol's solution (Text-Fig. IV, 1–3). This is the iodinophilous vacuole the content of which has been claimed by several observers as glycogen. In sections and smears the vacuole appears colourless and unstained.

Host-parasite Relations

1. *Infection.*—Attempts made to study how the infection of the fish takes place proved inconclusive. Great difficulty was experienced in getting the fish in the living condition and the few procured with difficulty did not survive for more than a few hours under Laboratory conditions. Treating the spores with the digestive juices extracted from various regions of the alimentary canal yielded only indecisive results. While in a few instances filament extrusion took place in about fifteen to twenty minutes, in no case was the sporoplasm seen to leave the spore cavity. Perhaps, this is due to the artificial conditions under which the experiments were carried out and it is probable that as in several other tissue infecting Myxosporidia, infection takes place by the ingestion of infected tissue containing spores by the host. The sporoplasm escapes from the spore cavity by the action of the digestive juices of the host and the liberated amœbula is carried through the blood stream to the primary site of infection.

2. *Autogamy and auto-infection.*—Almost all the writers have observed the copulation of the two germ nuclei in the sporoplasm, either before or immediately after the germination of the spore. This is clearly a case of autogamy. It has already been pointed out that a few spores were observed having only a single nucleus in the sporoplasm. Some of these spores were in an infiltrated condition, directly in contact with the host tissue. There are evidences to show that these spores germinate under favourable conditions and set up fresh infections in the same host. I have in my preparations young trophozoites having a single spore lodged in the cytoplasm, and with the two spore valves opened out (Photomicrograph 3). Since spore formation has not commenced in these forms, it is clear they are formed by the growth of the amœbulæ liberated from the spores under certain circumstances. This phenomenon of auto-infection partly accounts for the innumerable cysts seen in cases of heavy infections. Probable cases of

auto-infection in Myxosporidia have been described by Lieberkuhn (1854), Pfeiffer (1891), Thelohan (1895), Georgevitch (1914), Debaisieux (1922) and Kudo (1926). These writers hold that the spores could under favourable conditions, germinate setting up fresh infections.

3. *Pathogenesis.*—Histozoic Myxospordia have long been known responsible for some epidemics in fish which bring about a high rate of mortality among the hosts infected. The " boil disease " in the barbel and other fresh-water fishes of Europe is known to be caused by the infection of the muscle and connective tissue of the body-wall by *Myxobolus pfeifferi* (Keysselitz, 1908). Davis (1924) showed the cause of the " wormy " halibut along the Pacific coast as due to the presence of an intracellular myxosporidian *Unicapsula muscularis* within the mucsle fibres. The disease popularly known as the ' Drakrankheit ' bringing about a high rate of mortality especially in young fish of the Salmonidæ and the Gadidæ has been shown, through the efforts of Hofer and Plehn (1904, 1924) caused by a myxosporidian infecting the cartilage of the auditory organ.

There are no external pathological changes in the present instance to show infection. The vegetative forms grow between the muscle fibres in the wall of the bulbus and as their growth proceed the fibres turn through various angles from their original axial direction. The individual fibres get separated from one another and often present a frilled appearance. The muscle fibres immediately in contact with the parasite degenerate completely and their nuclei are hardly stained. When the vegetative forms are present in large numbers the destruction of tissue brought about is considerable. The fish reacts to the presence of the parasite by producing a number of active fibroblasts all round the parasite which give rise to several layers of connective tissue sheaths. This newly formed tissue completely shuts off the parasite from the muscle layer. The formation of a connective tissue cyst-wall seems to be induced by parasitic stimulation, a parallel to which is described by Mavor (1916) and Kudo (1929) in the case of the subdermal connective tissue of *Pimephalus notatus* infected by *Thelohanellus notatus* (Mavor), where an epithelial layer of cells is said to arise in the tissue immediately in contact with the parasite. Kudo describes this tissue as modified connective tissue cells of the host which modification took place as a result of parasitic stimulation. The muscle fibres of the barbel infected by *Myxobolus pfeifferi* are said to undergo hyaline degeneration leaving behind yellow granulations as degeneration products. The ' wormy ' halibut (Davis, 1924) shows swollen muscle fibres that undergo a hyaline degeneration, but in spite of the abundance of the parasite, no fibre is said to be entirely destroyed.

A great increase of blood capillaries is noted in the infected organ. When infection is heavy the bulbus appears swollen and reddish in colour. The blood capillaries are fully distended and an exudation of leucocytes takes place suggesting inflammation. Dark pigment granules appear all over the cysts and these are absent in healthy fish.

The infected fish appears to recover when only a few cysts are present. An active multiplication of connective tissue cells takes place round the parasite and this newly formed tissue penetrates the cysts after the death of the parasite. For a time the newly formed tissue and the spores intermingle. Later on, the spores degenerate completely, leaving behind a few dark staining bodies (Photomicrograph 5). In heavy infections the wall of the bulbus contains nothing but numerous cysts and a few strands of muscle in between the parasites. It is difficult to imagine that in such cases the host recovers. Pathological change of a different kind was observed in a few cysts. In these the parasites had apparently degenerated leaving behind a number of yellowish refractile concretions, the exact nature of which could not be determined (Photomicrograph 6). These bodies are perhaps degeneration products of the parasite or calcification products due to myxosporidian infection. The proper functioning of the organ is impaired in yet another way. The cysts often project into the lumen of the bulbus practically obliterating the blood passage. Under these conditions the normal functioning of the organ cannot take place.

It may be interesting to note here that in the many instances recorded, the high rate of mortality through myxosporidian infection is said to be more through secondary invasion by bacteria and fungi through the lesions produced by the parasite, than by the myxosporidian itself. In the present case the site of infection happens to be an internal organ and as such the chances of secondary infection are remote. It is therefore probable that the mortality rate amongst the infected fish may not be high. This fact could not be ascertained since the host happens to be marine not confined to a limited area as in some fresh-water fishes.

4. *Diffuse and scattered infiltrations.*—The phenomenon of diffuse infiltration or the intermingling of the host tissue with the spores of the parasite is of wide-spread occurrence in many tissue infecting Myxosporidia. The cysts under certain conditions rupture liberating the spores and developing pansporoblasts that are carried by the blood and lymph stream into the surrounding tissue spaces. The pansporoblasts complete their development and a condition is reached when the spores and tissue appear to intermingle.

Scattered spores away from the primary site of infection have been located in the connective tissue of the kidney. They are generally found encapsuled in sheaths in groups of two. The scattering of the spores appears to take place by the rupturing of the cysts located near the lumen of the bulbus. The spores and developing pansporoblasts carried by the blood stream get lodged in the kidney. The pansporoblasts perhaps complete their development in the new surroundings which accounts for the presence of spores in groups of two.

5. *Seasonal occurrence.*—The host fish are available practically throughout the year though found in large numbers from October to December. From March to July they are comparatively rare. The fish generally attains a length of about three feet. The larger specimens are not caught from the coastal region. Examination of fish of different lengths shows that fish measuring 6 to 10 inches are most susceptible to infection. Very young and full-grown fish are generally free from infection. Table I gives the result of the examination of only a representative collection of 150 fish caught at different times of the year and kept preserved in the Laboratory. It may be seen from the Table that about 75 per cent. of the medium sized fish are infected in different degrees.

TABLE I

Showing relation between size of fish and infection

Length of fish	Number of fish examined	Number infected	Nature of infection
Inches 2–6	42	3	Fairly heavy infection in one.
6–8	52	44	All grades of infection.
8–10	44	29	Majority heavily infected.
10–15	12	Nil	

The vegetative forms are found in fish examined in the colder months of the year from November to February. The temperature of the coastal waters ranges from 23 to 27 degrees Centigrade in these months. Fish examined in the remaining months of the year contain only ripe cysts with mature spores. The temperature of the sea-water ranges from 27 to 30 degrees during these months. Table II gives a record of the examination of *Otolithus ruber* during the different months of the year 1937. It may be noted that from April on there is a fall in the percentage of fish infected

TABLE II

Showing seasonal occurrence of stages of parasite in 1937

Months	Size of the fish	Number examined	Number infected	Stages of parasite	Nature of infection
	Small	8	1	Ripe cysts	Light
August ..	Medium	7	4	Ripe cysts	Fairly heavy
	Big	3	Nil	..	..
	Small	6	Nil	..	..
September ..	Medium	15	9	Ripe cysts	All grades
	..	..	..	..	..
	Small	9	1	Ripe cysts	Fairly heavy
October ..	Medium	22	16	Ripe cysts	All grades
	Big	6	Nil	..	..
	Small	10	2	Vegetative forms	Heavy
November ..	Medium	8	5		All grades
	..	..	..		..
	Small	5	Nil	..	..
December ..	Medium	12	9	Sporulating forms	All grades
	..	..	..	..	..
	Small	16	2	Sporulating cysts and vegetative forms	Fairly heavy
January ..	Medium	11	9		Mostly heavy
	..	..	..	..	..
	Small	5	Nil	..	..
February ..	Medium	16	11	Ripe cysts and few vegetative forms	Fairly heavy
	..	..	..	..	..
	..	..	..	..	..
March ..	Medium	8	6	Ripe cysts	Heavy
	..	..	..	..	..
	Small	11	2	Ripe cysts	Light
April-July ..	Medium	33	17	Ripe cysts	All grades
	Big	5	1	Ripe cysts	Light

and it is believed this fall is due to a certain amount of mortality among the infected fish in these summer months when the maximum temperature is reached. Keysselitz found that the " boil disease " in the barbel is not manifest in winter and spring months, but appeared in April, the maximum mortality being reached in the warmer months of the year. This author showed that sporulation is accelerated in fish kept in aquaria maintained at a temperature of 25 degrees Centigrade or higher. Nemeczek (1911) observed that after October, cysts of *Henneguya gigantea* do not contain spores but vegetative forms only. In *Henneguya similis* infecting the gills of *Perca fluviatilis* of Lake Constance, Zandt (1924) noted a periodic re-appearance of the parasite; the infection was first noted in December and January when mature spores are not found. The spores appear from February to March and infected fish were observed up to the end of May. From May to December no host fish carrying cysts were recognised. Keysselitz and Nemeczek agree that spore formation is influenced by the temperature of water in which the host fish live and both authors are of opinion that a higher temperature accelerates spore formation. The observation on *Henneguya otolithi* confirm the findings of the above authors.

Summary

1. A new species of a polysporous tissue infecting myxosporidian, *Henneguya otolithi* is described from the bulbus arteriosus of two species of a marine fish, *Otolithus ruber* and *O. maculatus*.

2. Two kinds of nuclei, the vegetative and generative, are present in the earlier stages of the parasite. The vegetative nuclei are not present in later stages.

3. The pansporoblasts originate by the copulation of anisogamous gametes.

4. Evidences of multiplicative reproduction by plasmotomy in young vegetative forms are seen.

5. Autogamy is present and instances of auto-infection have been observed.

6. The histopathological processes in the infected organ are described.

7. Diffuse infiltration of spores is common and scattered spores have been located in the kidney.

8. A seasonal variation in the occurrence of the different stages of the parasite is noted. In general a higher temperature seems to accelerate spore formation.

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EXPLANATION OF TEXT-FIGURES AND PLATE

All figures in the text were drawn with the camera lucida at stage level, with Zeiss apochromatic 120 oil immersion objective and compensating oculars. All figures, unless otherwise stated, are from sections of the bulbus arteriosus fixed in formol-sublimate-acetic, and stained with iron hæmatoxylin and eosin.

TEXT-FIG. I. A trophozotie where spore formation has not commenced. Note the clear differentiation of the ectoplasm and endoplasm. × 900.

TEXT-FIG. II. Part of a trophozoite highly magnified showing the vegetative and generative nuclei. × 1800

TEXT-FIG. III. Stages in spore formation, Figures 22 to 25 are from smears fixed in Schaudinn's fluid and stained with Giemsa. × 1800.

TEXT-FIG. IV. (1) Spore treated with dilute caustic potash solution. Note the extruded polar filament. (2) Spore treated with Lugol's solution showing the iodinophilous vacuole. (3) Side-view of a fresh spore to show the thick sutural ridge.

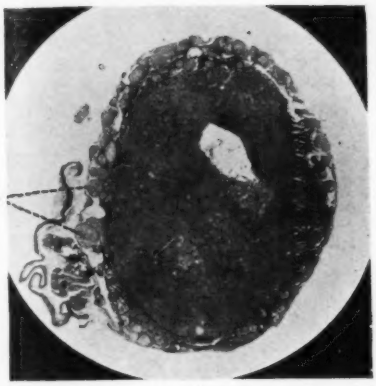
PLATE VII

PHOTOMICROGRAPHS 1 TO 6

1. Section of bulbus showing a large number of rounded cysts. × 150 approx.
2. Three trophozoites in section, with thin muscle strands of the host separating them. × 1350 approx.
3. A trophozoite by auto-infection. Note the single spore in the cytoplasm with the spore valves opened out. × 1800.
4. A ripe cyst showing mature spores. × 300 approx.
5. An infected area showing a late stage of fibrosis. Note the newly formed fibrous tissue and the darkly stained degenerate remains of the parasite. × 1350 approx.
6. Section passing through a number of cysts two of which contain dark concretions.

KEY TO LETTERING

cal. con. .. Calcareous concretions
cys. .. Cysts.
d. b. .. Dark bodies.
div. g. n. .. Dividing generative nucleus.
env. n. .. Nucleus of enveloping membrane.
gen. n. .. Generative nucleus.
ger. n. .. Germ nucleus.
iod. vac. .. Iodinophilous vacuole.
n. p. c. .. Nucleus of polar capsule.
pans. .. Pansporoblast.
pol. cap. .. Polar capsule.
pol. fil. .. Polar filament.
sp. val. .. Spore valves.
sut. rid. .. Sutural ridge.
tr. rid. .. Transverse ridge.
val. n. .. Valve nucleus.
veg. n. .. Vegetative nucleus.



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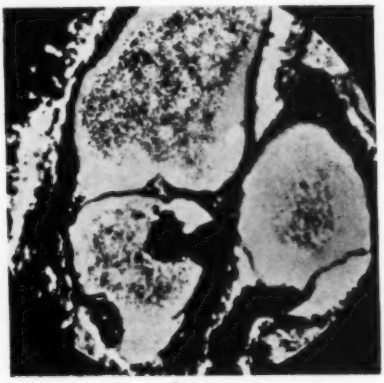

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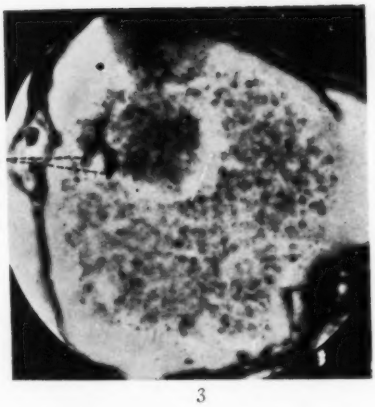

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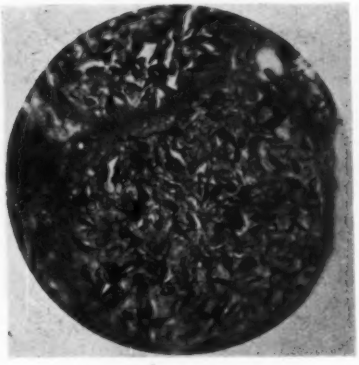

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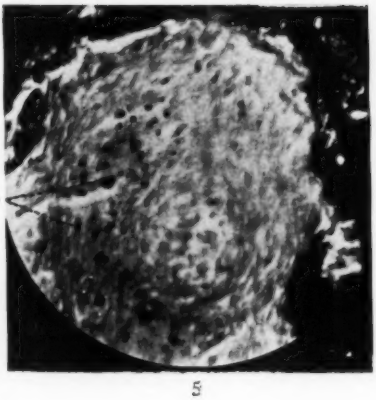

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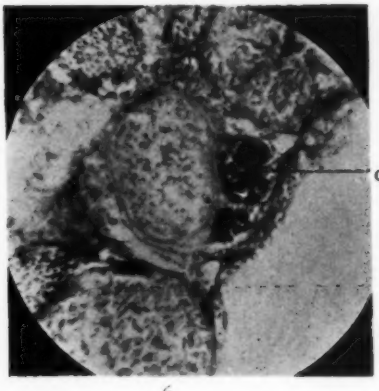

6

A LIST OF HOSTS OF SOME PHANEROGAMIC ROOT-PARASITES ATTACKING ECONOMIC CROPS IN INDIA

By L. S. S. Kumar and S. Solomon

(From the Department of Botany, College of Agriculture, Poona)

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During the last eight years, investigations have been in progress in the Economic Botanist's section, on phanerogamic root-parasites with particular reference to *Striga* and *Orobanche*. Early in the investigations on *Striga* it was found necessary to prepare a list of all the hosts on which the parasite lives in the absence of the cultivated hosts and compare it with the list of hosts published by previous workers. Only two such lists have appeared so far—one by Sawyer[8] in Burma and another by Saunders[7] in South Africa. Both these authors have worked on only one species namely *Striga lutea* and have given a list of its hosts as determined locally. Among the Indian publications on this subject, the papers by Van Buuren,[2] Luthra[6] and Barnes[1] may be mentioned.

Several requests for a list of the hosts of different species of root parasites have been received and it is felt that a list for India, although not complete, will partially fulfil the requirements of other workers on this subject. An attempt has been made to bring the following list as up-to-date as possible by including in it both the previous lists for *S. lutea* and any other host species mentioned by the Indian works referred to above. Wherever possible the author first recording the fact of the parasitism of the host species has been named.

Parasite	Host	Authority	
		Foreign	Indian
Striga lutea, Lour	*Andropogon annulatus* Forsk.	Sawyer	
	Andropogon caricosus, Linn.	Sawyer	
	Andropogon monticola, Schult.		Kumar and Solomon
	Andropogon sorghum, Hack.		Mollison
	Andropogon sudanense, Leppman and Bossmann		Chibber
	Aristida adscensionis, Linn.	Sawyer	
	Avena sativa, Linn.	Saunders	
	Brachiaria distachya, Haines		Coimbatore Agricultural Institute
	Celosia argentia, Linn.		Kumar and Solomon
	Chloris gayana, Kunth.	Saunders	
	Chloris virgata, P. Durand	Saunders	
	Commelina Hasskarli, Clarke		Kumar and Solomon
	Corchorus fascicularis, Lambk.	Sawyer	
	Cynodon dactylon, Pers.	Sawyer	
	Cyperus rotundus, Linn.	Sawyer	
	Dactylis glomerata, Linn.	Saunders	
	Digitaria sanguinalis, Scop.	Saunders	
	Echinochloa frumentacea Link.	Saunders	
	Echinochloa crus-galli, Beauv.	Saunders	
	Eleusine ægyptiaca, Desf.	Sawyer	Kumar and Solomon
	Eleusine coracana, Gaertn.		Millets Section, Coimbatore
	Eragrostis abyssinica	Pearson	
	Eragrostis spp., Host.		Van Buurren
	Eriochloa polystachya, Duthie.	Sawyer	
	Euchlena mexicana, H.B.K.	Sawyer	
	Eurochloa helopis	Saunders	
	Hordeum intermedium, Keke.	Saunders	
	Indigofera glandulosa, Wild.		Kumar and Solomon
	Imperata arundinaceæ, Cyrill	Palm and Heusser	

Parasite	Host	Authority	
		Foreign	Indian
Striga lutea, Lour	*Ipomœa reniformis*, Chois	Sawyer	
	Oryza sativa, Linn.		Barber
	Panicum brizanthum, Hochst.	Saunders	
	Panicum coloratum Kunth.	Saunders	
	Panicum colonum Linn.	Sawyer	
	Panicum distachyum, Linn.	Sawyer	
	Panicum flavidum Retz.	Sawyer	
	Panicum Isachne, Roth		Kumar and Solomon
	Panicum maximum, Jacq	Saunders	
	Panicum miliaceum, Linn.	Saunders	
	Panicum miliare, Lamk.	Sawyer	Kumar and Solomon
	Panicum prostratum, Lamk.	Sawyer	
	Panicum repens, N.L.B.	Sawyer	
	Paspalum dilatatum, Linn.	Pearson	
	Paspalum scrobiculatum, Linn.		Chibber
	Paspalum virgatum, Linn.	Saunders	
	Pennisetum typhoideum, Rich.	Sawyer	Kumar and Solomon
	Pennisetum unisetum, Benth.	Saunders	
	Saccharum officinarum, Linn.	Pearson	Mollison
	Secale cereale, Bieb.	Saunders	
	Setaria gerrardii, Stapf.	Saunders	
	Setaria italica, Beauv.	Sawyer	Kumar and Solomon
	Setaria lindenbergiana, Stapf.	Saunders	
	Setaria nigrirostris, T. D. and Schizz	Saunders	
	Sporobolus coromandelianus, Link.	Sawyer	
	Tribulus terrestris, Linn.	Sawyer	
	Triticum vulgare, Host.	Saunders	
	Zea Mays, Linn.	Fuller	Kumar and Solomon
Striga densiflora, Benth.	*Andropogon contortus*, Linn.		Van Buuren

Parasite	Host	Authority	
		Foreign	Indian
Striga densiflora, Benth.	*Andropogon pumilus*, Roxb.		Kumar and Solomon
	Andropogon sorghum, Hack.		Van Buuren
	Commelina Hasskarlii, Clarke		Kumar and Solomon
	Cyperus spp., Linn.		Kumar and Solomon
	Desmodium diffusum, DC.		Kumar and Solomon
	Digitaria Royleana, Prain.		Kumar and Solomon
	Eleusine ægyptiaca, Desf.		Kumar and Solomon
	Eragrostis spp. Beauv.		Van Buuren
	Euchlæna mexicana, Schrad.		Kumar and Solomon
	Glossocardia linearifolia, Cass.		Kumar and Solomon
	Indigofera cordifolia, Heyne		Kumar and Solomon
	Iseilema laxum, Hack.		Kumar and Solomon
	Iseilema wightii, Andrews		Barnes
	Lophopogon tridentatus, Hack.		Kumar and Solomon
	Panicum Isachne, Roth.		Kumar and Solomon
	Paspalum sanguinale, Lamk.		Kumar and Solomon
	Paspalum scrobiculatum, Lamk.		Kumar and Solomon
	Pennisetum typhoideum, Rich.		Kumar[5]
	Saccharum officinarum, Linn.		Luthra
	Setaria glauca, Beauv.		Kumar and Solomon
	Setaria italica, Beauv.		Kumar and Solomon
	Tragus racemosus, Scop.		Kumar and Solomon
	Tripogon Jacquemonti, Stapf.		Kumar and Solomon
Striga euphrasioides, Benth.	*Andropogon contortus*, Linn.		Kumar and Solomon
	Andropogon sorghum, Hack.		Luthra
	Aristida funiculata, T. and R.		Kumar and Solomon
	Cyperus spp., Linn.		Kumar and Solomon
	Digitaria Royleana, Prain.		Kumar and Solomon
	Eragrostis cynosuroides, Beauv.		Kumar and Solomon

Parasite	Host	Authority	
		Foreign	Indian
Striga euphrasioides, Benth	*Oldenlandia aspera*, DC.		Kumar and Solomon
	Oryza sativa, Linn.		Barnes
	Panicum colonum, Linn.		Kumar and Solomon
	Panicum ramosum, Linn.		Kumar and Solomon
	Polygala erioptera, DC.		Kumar and Solomon
	Saccharum officinarum, Linn.		Barber
	Spermacoce stricta, Schlecht.		Kumar and Soloman
	Sporobolus diander, Beauv.		Kumar and Solomon
	Zea Mays, Linn.		Kumar and Solomon
Striga orobanchoides, Benth.	*Dysophylla quadrifolia*, Benth.		Barber
	Euphorbia antiquorum, Linn.	Trimen	
	Hygrophila seryphyllum, Andrews		Van Buuren
	Lepidagathis cristata, Willd.		Van Buuren
Sopubia delphinifolia, G. Don.	*Anthistiria ciliata*, Linn.		Kumar and Solomon
	Chrisopogon montanus, Trin.		Barnes.
	Peltophorus divergens, Camus		Kumar and Solomon
	Andropogon sorghum, Hack.		Kumar[4]

The above list gives 54 hosts of *S. lutea*, of which only 18 have been recorded in India. The authors have contributed five new hosts to this list.

Of the list of 24 hosts of *S. densiflora*, the authors are responsible for 18. Of the 15 hosts of *S. euphrasioides*, 12 have been recorded by the authors for the first time. *S. orobanchoides* has only four hosts to its credit, all of which have been mentioned previously. The authors have contributed two new hosts of the four recorded for *Sopubia delphinifolia*.

Saunders[7] believes that non-graminous plants cannot be hosts of *S. lutea*. It is not clear how he came to this conclusion. But Sawyer[8] has listed five non-graminous hosts and the authors record three more. It would appear that the presence of members of the Graminæ is only necessary for the germination of *Striga lutea* seeds ; once they have germinated they can attack other host roots besides those of the Graminæ. This is a tentative conclusion as

the host list was prepared after collecting the parasites together with their immediately neighbouring hosts along with a clod of earth ; the roots were then gently washed in water and whenever a swelling characteristic of the point of haustorial connection was found, the host was preserved for identification later. Herbarium specimens of several hosts of all the species of *Striga* have been prepared and preserved in this manner.

Barnes,[1] in stating that according to Gamble,[3] *S. densiflora* is not recorded as parasitic, records one case of parasitism in this species. During the past eight years work, the parasitism of *S. densiflora* on the hosts given in the above list has been confirmed over and over again. It may also be noted that Tadulingam and Venkatanarayan[9] in their book on South Indian weeds mention only *S. lutea* and *S. euphrasioides* as parasitic and have omitted *S. densiflora* altogether.

The investigations which began in 1932 are being financed by the Imperial Council of Agricultural Research since May 1938.

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STUDIES IN *SORGHUM HALEPENSE* (LINN.) PERS—THE JOHNSON GRASS*

By G. N. Rangaswamy Ayyangar, F.N.I., I.A.S.,
Millets Specialist and Geneticist
AND
B. W. X. Ponnaiya, B.Sc. Ag.,
Assistant, Millets Breeding Station, Coimbatore

Received January 30, 1941

Sorghum halepense is the earliest known wild sorghum. It has been recorded as early as 1753 as *Holcus halepensis*. The origin of the word *halepense* is from Aleppo, a town in Syria, where the grass was first found and described[1].

This grass is a native of the Mediterranean coastal countries of Europe, Africa and Asia and extends eastwards through Arabia to India. It was first introduced into America in 1830 as a fodder grass by "Governor Means of S. Carolina". Ten years later Col. William Johnson, the owner of a large plantation introduced it in the rich "bottom lands" of the Alabama river. From here it was distributed all over America and hence it is called 'Means on Johnson Grass.'[2]

The grass came up well wherever it was grown. However it soon turned out to be somewhat of a pest on account of its perennial underground stems, which made it very difficult to eradicate, once it was established. Because of this undesirable feature a world search was made by the American Botanists for a better fodder grass and this resulted in the introduction of the Sudan grass (*S. sudanense*) into America and subsequently into Australia.[3]

S. halepense belongs to section *Eu-sorghum* and sub-section *Halepensia* (Snowden).[4] The distinctive features of this group are the presence of rhizomes and a diploid number of 40 chromosomes. The only known species of this sub-section is *S. halepense*, but Snowden is of the opinion that one or two other species also may be found to belong to this group.

At one time this grass was considered to be the original ancestor of the cultivated sorghums. However after the discovery of many other wild sorghums and after studying their inter-relations with the cultivated ones,

* This is the second in a series of articles on the Sorghum groups. The first one on *S. sudanense* appeared in the *Proc. Ind. Acad. Sci.*, 1939, 10, 237–54.

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the latest opinion is that *S. halepense* could not be the progenitor of the cultivated sorghums. The main reasons for this are firstly the higher chromosome number (*viz.*, 40 against 20) and its inability to cross readily with the cultivated sorghums, and secondly the presence of rhizomes.[4]

Apart from its systematic and economic aspects, very little study has been done on this species. This paper presents some of the observations made on samples, both Indian and foreign, grown at the Millets Breeding Station, Coimbatore, during the last ten years. The Indian samples include six from the Madras Presidency, one from Hyderabad (Deccan), and one from Indore. The foreign samples are represented by one sample from Palestine and two through Germany.

Agro-Botanical Description[5]

Duration—100 to 165 days; *Seedlings*—coleoptile deep purple, purple or green; leaves, bluish green; habit, open; *Nodal band*—hairy, purple or green; *Leaf-sheath*—blackish purple; *Leaf-blade*—margin flat[6]; *Leaf-midrib*—white, edges hairy[7]; *Auricular junction*—purple or green; *Axil of leef-sheath above the nodal-band*—purple or green; *Panicle*—well emerged, above 20 cm. from the subtending leaf, penduncle about 50 cm. long. Loose, conical, primary, secondary and tertiary branches pulvinate and horizontal; *Spikelets*—cymosely arranged, hairy; deciduous; *Stigma*—purple, light purple or light yellow; *Anther*—(fresh) purple or light yellow; (dry) lightbrown ; *Awn*—9 to 11 mm., sometimes 0; *Grain*—brown in colour enclosed in glumes; *Glumes*—bleached blackish purple or unbleached; *Tillers*—15 to 20, un-uniform maturing type; *Bloom*—a thin coating of bloom on leaf-sheaths and internodes which is very marked when grown under dry conditions.

General Characters

This grass is found in abundance in the Eastern and Western ghats and also along the river banks in South India. On the hills it is distributed in dry places as well as near water courses. In dry places the grass does not come up luxuriantly although it persists tenaciously. It dominates over all other grasses when found near water courses.

There are two distinct types with regard to duration. The long duration ones take 150 to 165 days from the date of sowing for the first set of panicles to ripen. The plants are larger, the average height being about 250 cm. The internodes are about 0·8 cm. thick and the average length of panicle is 40 cm. These flower only during November and December (season-bound). The short duration types take from 100 to 120 days for the first set of panicles to reach maturity. The plants are smaller, their average

height being only 150 cm. The internodes are 0·6 cm. thick and the average panicle length is 25 cm. This variety is period-bound and flowers in 2½ to 3 months after sowing. The tillering capacity is similar in both the cases.

The rhizomes are formed only after the flowering of the tillers. At this stage some of the axillary buds of the basal internodes below the soil level become geotropic and this is the beginning of the rhizome formation. The growing point of the rhizome is well protected by scale-leaves which consist mostly of modified leaf-sheaths. In stray cases rudiments of the leaf-blades also are found. As this stem grows under the soil, roots are developed at every node. The rhizomes branch further and spread along the sides forming a mat under the soil (Fig. 2). If by chance any growing point gets outside the soil, it at once turns into an aerial stem. The rhizomes are very brittle at the joints. The broken bits can be dormant for a whole year and shoot up during the rains of the succeeding season.

Due to their early flowering, short duration varieties develop rhizomes much earlier and more profusely than the long duration ones. In the latter the rhizomes commence to devlop only late (during November and December) and the development is not profuse unless there is a good supply of water. Thus it will be seen that the short duration varieties can persist even under dry conditions whereas the long duration ones can flourish only under swampy conditions.

The main shoot is the first to flower followed by the successive flushes of primary, secondary and tertiary tillers. In the later tillers the height gets reduced and the panicles tend to become smaller. The anthesis in both the long and short duration types occurs between 8–30 and 9–30 A.M.[8] The order of anthesis is similar to that of the cultivated sorghums.[9] The grain attains the *dough* stage within 3 to 4 weeks after flowering. The spikelets commence shedding even in this unripe condition. All the pedicelled spikelets are deciduous. In the case of the sessile spikelets, the basal ones get shed whereas those placed towards the tips of panicle branches persist. These form about 10 per cent. of the sessile spikelets. The seeds require a resting period of one year for good germination. Even after this resting period it is seen that only a third of the seeds germinate at first and the rest germinate slowly in successive waves along with irrigation or rains.

As a fodder, though a bit coarse, it gives an excellent yield when grown on fertile lands and river bunds. Under dry conditions it does not give a good yield although it is able to withstand drought. Its perennial habit makes it an undesirable one in crop rotation. Like the other sorghums this also contains a cynogenetic glucoside in the early stages.

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Inheritance of Characters

(*a*) *Purple Colour on Stigmas.*—The stigmatic colour in *S. halepense* is usually purple although variations upto yellow are also met with. Occasionally this yellow exhibits a light tint of purple at the ends of stigmatic feathers. The F_1 generation of a cross between purple and yellow was a plant with purple stigmas. The F_2 gave 28 plants with purple stigmas and eight plants with yellow stigmas indicating a single factor difference. The F_3 generation confirmed this behaviour (188 purple, 60 yellow). A similar experience has been recorded in the cultivated sorghums also.[10]

(*b*) *Reduced Filaments.*—In 1935 a thin-stemmed plant, probably a mutant, was observed in a pure line from Palestine. In the next season this plant gave a segregation for normal anthers (28) and non-emergent anthers (10) at the time of anthesis. This abnormal condition was found to be due to a reduction in the length of the filaments. The distinctive characters between these two types of plants are presented in the following table:—

Character	Normal anthers	Reduced filamented anthers
Filament length ..	Long 4·0 mm.	Short 0·5 mm.
Position of anthers at the time of anthesis ..	Dangle out	Stuck up within the spikelet
Size of anthers	3·0 × 1·0 mm.	3·0 × 0·75 mm. narrower than the normal
Sterile pollen	0 to 2%	90 to 100%
Stigmas	Widely divergent after anthesis	The stigmatic brushes come out but are near each other

In the reduced filamented plants the anthers dehisce only rarely and when selfed only stray seeds get set (less than 1%). The stigmas however are fertile as shown by the high percentage of natural crosses.

The time of anthesis of the reduced filamented plant is the same as that of the normal plant (8–30 A.M.). At that time the glumes gape out and expose the three anthers and the stigmatic brushes. As the filaments are short the anthers remain rigid and erect. The stigmas are caught up between the anthers and only the tips manage to show themselves out at the top of the glumes when the latter close up.

Six selections were carried forward from this F_2 generation and their behaviour is given below:—

Selection number	Character of the F_2 selection	F_3 behaviour	
		Normal anthers	Reduced filamented anthers
S. 272	Reduced filamented anthers	..	Pure
S. 273	" " "	..	"
S 270	Normal anthers	Pure	..
S. 268	" "	80	30
S. 269	" "	68	22
S. 271	" "	37	11
	TOTAL	185	63

The above segregations show that the reduced filamented condition is a monogenic recessive to the normal.

(*c*) *Compact Panicle*[3,11].—S. 164 is a pure line from the Godavari delta, South India. In this lot two mutants occurred in the year 1937 in a population of 25 plants. Both of them had compact panicles. The following tabular statement gives the differences between the normal and the mutant plants:—

Character	Normal	Mutant
Height	250 cm.	150 cm.
Number of tillers	10 to 15	1 to 2
Panicle—length of central axis	35 to 45 cm.	15 to 25 cm.
" " primary branches	20 to 25 cm.	7 to 10 cm.
" " secondary branches ..	5 to 10 cm.	1 to 3 cm.
" " tertiary branches	1 to 4 cm.	Absent
" shape	Loose conical	Compact, rod-like
Pulvinus	Present	Absent

From the above Table it will be seen that in the mutants there is a reduction in the length of the central axis and the side branches of the panicle. The tertiary branches are absent. The tillering capacity is distinctly reduced and the plants are shorter (Figs. 4 and 5).

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In the first mutant the spikelets were normal. This was grown in the next season as S. 267, and in this pure breeding progeny a natural cross with loose panicle was noted. This natural cross when sown gave 28 plants with loose conical panicles and 8 plants with compact panicles, indicating that compactness is a monogenic recessive to the loose panicle.

The second mutant was similar to the previous one but the spikelets appeared stouter due to the extra-fertility of the lower floral glume. The seed-setting however was poor and so the type could not be propagated although it bred true.

(*d*) *Triple Stigmas*.—In some pure lines stray plants with odd ovaries with triple stigmas were met with (Fig. 3). Subsequent tests proved that this abnormality was not heritable.

Summary

A brief description of *S. halepense* and its relationship to the cultivated sorghums is given. The formation of rhizomes has been described. The inheritance of a few characters has also been recorded. Purple colour in the stigma is a monogenic dominant over the non-pigmented condition (yellow). Reduced filament is a simple recessive to the normal. The compact panicle was found to behave as a monogenic recessive to the loose panicle. Stray cases of extra-fertility of spikelets and ovaries with triple stigmas have been found to occur.

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FIG. 1
Entire plant

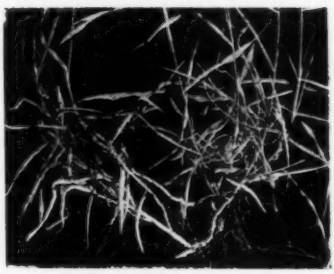

FIG. 2
Rhizomes

FIG. 3
Triple stigma

FIG. 4
Normal and Compact panicled plants

FIG. 5
Normal and Compact panicles

Sorghum halepense

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TWO NEW NEMATODES FROM AN AQUATIC BEETLE

By M. A. Basir

(Department of Zoology, Muslim University, Aligarh, U.P., India)

Received January 18, 1941

(Communicated by Dr. M. B. Mirza)

The nematodes described in this paper were collected by Mr. M. G. H. Beg of Srinagar College, Kashmir, from the aquatic beetles inhabiting the hill streams of Islamabad, a town in Kashmir State at a height of about 6,000 feet above sea level. Unfortunately the beetles from which the material was collected were lost by him and so the exact name of the host could not be given in this paper. However, the writer has requested Mr. Beg to collect them again for identification of the host. But as up to this time he could not do so, the writer thinks it necessary to publish the account of the parasites. The material contains two species, both of which belong to the sub-family Thelastomatinæ. They appear to be new, and represent a new genus for which the name *Galebiella* is proposed.

Genus Galebiella gen. nov.

Generic Diagnosis.—Thelastomatinæ.

Male unknown.

Female with narrow lateral alæ. Head distinctly set off as several modified annules; oral opening surrounded by eight sub-median papillæ. Buccal cavity short and cylindrical. Oesophagus consisting of an anterior corpus terminated by an oval swelling, a distinctly set off isthmus, and a posterior valvular bulb. Excretory pore not observed. Intestine dilated anteriorly to form a cardia; diverticula absent. Tail conical. Vulva about two-third of the body length from the anterior end. Vagina directed anteriorly. Uteri divergent. Eggs oval.

The genus *Galebiella* appears to be most closely related to the genus *Leidynemella* Chitwood, 1934, but differs from the latter in several important characters. In *Galebiella* the swelling at the base of the corpus is oval rather than sub-spherical. The position of vulva in *Leidynemella* is described by Chitwood, 1934 as " approximately half way from head to anus " while in *Galebiella* the vulva is distinctly posterior; it is two third of the body length (with tail) from the anterior end. Vagina in *Galebiella* is directed

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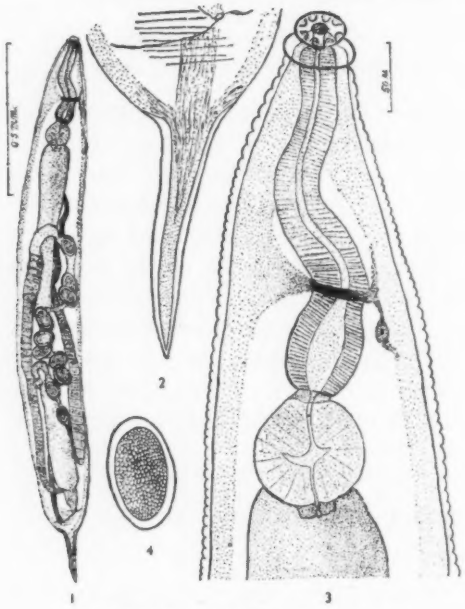
anteriorly instead of posteriorly as in *Leidynemella*. In *Galebiella* tail is conical while in *Leidynemella* it is described as filiform. Because of these differences the writer has thought it appropriate to create a new genus for the worms described in this paper.

Type Species.—Galebiella galebiella gen. et. sp. nov. (Figs. 1 to 4).



Galebiella galebiella gen. et sp. nov.

FIG. 1. Female, entire, latero-ventral view.
„ 2. Female, tail, latero-ventral view.
„ 3. Female, œsophageal region.
„ 4. Egg.

Specific Description.—Galebiella:

Male unknown.

Female, 1·85 mm. long by 210 μ wide. Head set off as two modified annules, measuring 15 μ and 20 μ in width respectively. Body striated; anterior striations being more distinct. Average width of striations about 8 μ. Lateral alæ present but narrow. Oral opening surrounded by eight papillæ. Buccal cavity cylindrical, 13 μ deep by 8 μ wide. Oesophagus 360 μ long, anterior part of corpus 200 μ long by 40 μ wide, posterior part presents an ovoid swelling 65 μ long by 55 μ wide, isthmus 10 μ long by 25 μ wide distinctly set off, bulb 80 μ long by 85 μ wide. Nerve ring 200 μ

from the anterior end of body. Excretory pore not observed. Anus 255 μ from the posterior end of body. Tail conical. Vulva two-third of the body length from the anterior end, 1·17 mm. from the anterior end of body. Vagina directed anteriorly; amphidelphic. Eggs oval, 85 μ long by 50 μ wide.

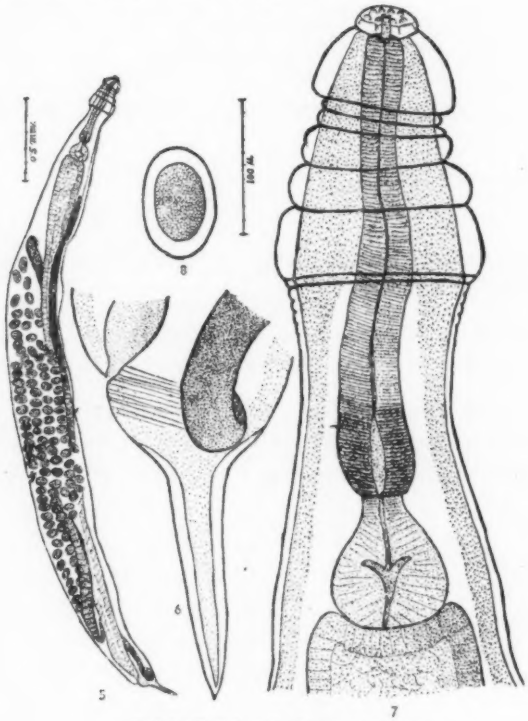
Host.—Aquatic beetle.

Location.—Presumably intestine.

Type Locality.—Mountain springs of Islamabad, Kashmir, (height above sea level, about 6000 ft.).

Type Specimen.—Museum of the Zoological Laboratories, Muslim University, Aligarh; Helminthological collection No. 1021.

Galebiella islamabadi sp. nov. (Figs. 5 to 8).



Galebiella islamabadi gen. et sp. nov.

FIG. 5. Female, entire, lateral view.
„ 6. Female, tail, lateral view.
„ 7. Female, œsophageal region.
„ 8. Egg.

Specific Description.—Galebiella:

Male unknown.

Female, 4·3 mm. long by 375 μ wide. Narrow lateral alæ present. Head distinctly set off as seven big annules, first annule 60 μ, second and third 12 μ, fourth 30 μ, fifth 35 μ, sixth 55 μ, and eighth 8 μ wide respectively. Behind these annules the cuticle presents about four faint striations each 8 μ wide. The rest of the body is not striated at all. Oral opening surrounded by eight papillæ. Buccal cavity cylindrical, 18 μ deep by 8 μ wide. Oesophagus 450 μ long, anterior corpus 285 μ long by 45 μ in maximum width, posterior part presenting an ovoid or sub-spherical swelling 65 μ long by 55 μ wide, a short isthmus 15 μ long by 30 μ wide distinctly set off, posterior valvular bulb 85 μ long by 90 μ wide. Nerve ring and excretory pore not observed. Intestine dilated anteriorly to form a slight cardia. Anus 260 μ from the posterior end of body. Tail conical. Vulva about two third of the body length from the anterior end, 2·6 mm. from the anterior end of body. Vagina directed anteriorly; amphidelphic. Eggs oval, 82 μ long by 55 μ wide.

Host.—Aquatic beetle.

Location.—Intestine.

Type Locality.—Mountain springs of Islamabad, Kashmir, (height above sea level, about 6000 feet.).

Type Specimen.—Museum of the Zoological Laboratories, Muslim University, Aligarh; Helminthological collection No. 1022.

This species differs from *Galebiella galebiella* in size of body, in shape, number and size of head annules, in striations on the body, in the form and size of the posterior corpus swelling, and in the shape and size of eggs.

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COMMELINA ALISAGARENSIS KUMAR AND DEODIKAR: A NEW SPECIES FROM HYDERABAD DECCAN, INDIA

By L. S. S. Kumar and G. B. Deodikar

(From the Department of Botany, College of Agriculture, Poona)

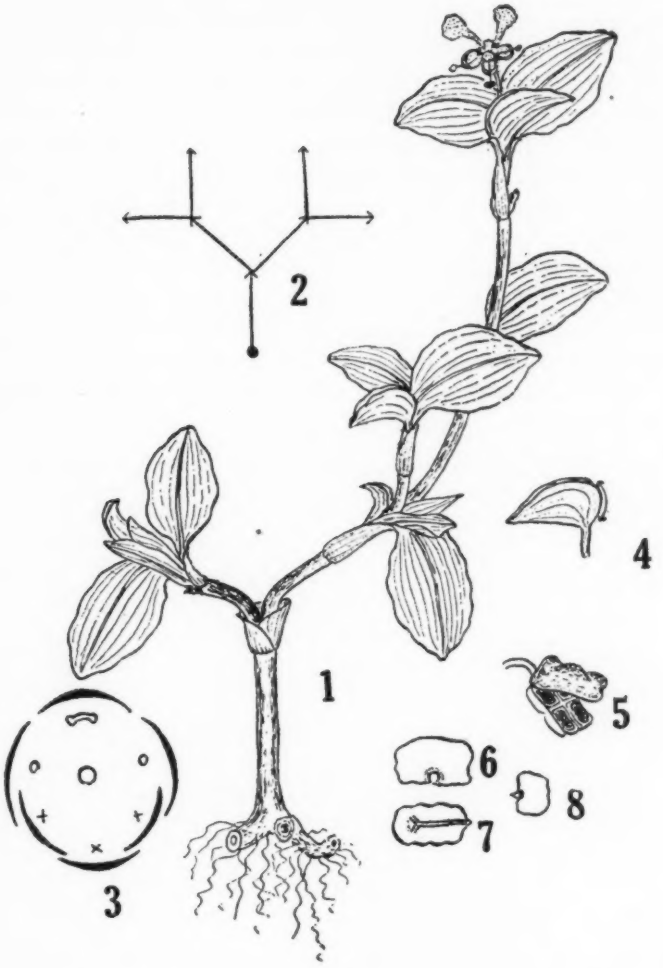
Received January 28, 1941

The senior author during his tour in Hyderabad State visited Alisagar, a small hill resort in the Nizambad district. The new species (*C. alisagarensis*, Kumar and Deodikar) was found growing at an altitude of about 2,000 feet. The distribution of the new species appears to be restricted since from several other places on the plains round about Alisagar only other species of *Commelina* were collected but not this particular one. The authors sent specimens to the Royal Botanic Gardens, Kew, for verification and the species is now confirmed to be new and not previously described.

The authors are indebted to Mr. H. K. Airy Shaw for the latin description and to the authorities of the Royal Botanic Gardens, Kew, for help in identification.

Commelina alisagarensis, Kumar and Deodikar, sp. nov.

Herb erect, 6″ to 12″ height. *Roots* fibrous, not bearing any cleistogamous flowers. *Stem* slender, glabrous, dichotomous-like branching. *Leaves* pubescent, elliptic with rounded apex, 1″ to 1½″ by ¾″ to 1″. *Sheaths* about ½″ long with margins ciliated and free. *Spathes* axillary, clustered, funnel shaped or cucullate, pubescent, apex acute, base auricled on both sides and loosely articulated along the lower portions of the margins, about ¾″ long and ½″ broad. *Peduncles* ¼″ to ⅛″ long. *Pedicels* 3 to 4 in number, included in spathes. *Sepals*, 3 in number, greenish blue, membranous, two internal and round, one external and ovate acute. *Petals* blue, 3 in number, 2 broad with long claw, one small with short claw. *Stamens* 3, two anthers small and round, one anther large and crescent shaped. *Staminodes*, 3 in number. *Ovary* trilocular. *Capsule* pubescent, thin walled, five seeded, two anterior cells each two seeded with seeds free, dehiscence loculicidal. *Posterior cell* one seeded, indehiscent with seed imperfectly formed, infertile and smaller than the seeds in the anterior cell of the capsule. *Seeds* deep black, smooth, oval, truncate on both sides with a white linear appendage, micropyle prominent.



Commelina alisagarensis, Kumar and Deodikar

1. Sketch of the whole plant, ¼ natural size. (2) Schematic representation of the mode of branching. (3) Floral diagram. (4) Spathe. (5) Capsule. (6) Seed, dorsal side with micropyle. (7) Ventral side with the white linear appendage. (8) Lateral view of seed. (5 × ½; 6, 7 and 8 × 4).

Herba erecta, 15–30 cm. alta. *Radices* fibrosæ, flores cleistogamos haud gerentes. *Caulis* gracilis, glaber, dichotome ramosus. *Folia* elliptica, apice rotundata, 2·5–3·75 cm. longa, 1·9–2·5 cm. lata, pubescentia. *Vaginæ* circiter 1·25 cm. longa, marginibus ciliatis liberis. *Spathæ* axillares, fasciculatæ, infundibuliformes vel cucullatæ, pubescentes, apice acutæ, basi utrinqe auriculatæ atque secundum partem marginum inferiorem laxe articulatæ. 1·9 cm. longæ, 1·25 cm. latæ. *Pedunculi* 3–4, in spathis inclusæ. *Sepals* 3, virescenti-cærulea, membranacea, duobus interioribus rotundatis, tertio exteriore ovato acuto. *Petala* 3, cærulea, duobus latis ungue longo, tertio parvo ungue brevi. *Stamina* 3, antheris duobus parvis rotundatis, tertio magno crescentiformi. *Staminodia* 3. *Ovarium* triculare. *Capsule* pubescens, pariete tenui, pentasperma, loculis 2 anticis loculicide dehiscentibus utrisque 2-spermis seminibus liberis, loculo posticio indehiscente 1-spermo, semine imperfecto sterili quam semina in loculis anticis minore. *Semen* saturate nigrum, læve, ellipsodeum, utroque latere truncatum ac appendice alba lineari præditum, micropyle prominente.

Alisagar, Nizambad District, Hyderabad State, Plains of Godavari River, South India. Collected on 12th August 1939.

STUDIES ON THE COMPARATIVE ANATOMY OF THE TAIL IN SAURIA AND RHYNCHOCEPHALIA

I. *Sphenodon punctatus* Gray

By Syed Muzammil Ali, M.Sc.

(*Department of Biology, St. Andrew's College, Gorakhpur, U. P.*)

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I. Introduction

In November 1938, Professor W. P. Gowland of the University of Ontario (Dunedin, New Zealand) presented Mr. Beni Charan Mahendra with a

full-grown specimen[1] of the Tuatara lizard, *Sphenodon punctatus* Gray, with a part of its tail regenerated. As this herpetological rarity is supposed to exhibit many primitive features in its anatomy, Mr. Mahendra very kindly entrusted it to me and suggested that I should study the structure of its tail in detail in order to see whether it throws any light on the evolution of the autotomous type of tail.

Although much excellent work has already been done on caudal autotomy and regeneration, no *comparative* study has so far been made of the structure of the tail in Sauria and Rhynchocephalia. Without a detailed knowledge of the anatomy of both the autotomous and the non-autotomous types, it is hardly possible to understand how the structural modifications requisite for autotomy came to be evolved. The present investigation has been undertaken, in the first place, to supplement the already existing knowledge on the subject, and secondly, to trace the evolution of the autotomous type of tail from the non-autotomous.

The present part of the series deals with the tail of *Sphenodon*, while the subsequent parts will take up the study in various representatives of Sauria, both autotomous- and non-autotomous-tailed, to be followed by a general discussion on the subject based on the data discovered.

II. Historical Résumé

As a résumé on the caudal autotomy and regeneration in Lizards will be given in a subsequent part, we are concerned here with the previous work on the tail of *Sphenodon* only.

Günther (1867) was the first to describe the peculiar structure of the caudal vertebræ in a general account of the anatomy of the Tuatara. Gadow (1896) particularly noted the position of the sub-division line in the caudal vertebræ in relation to the neural arch and transverse processes. Goette (1897) compared and contrasted the structure of the caudal vertebræ, with those of the trunk, pointing out that in the former (unlike the latter) the vertebral canal is most spacious within the vertebra and narrowest at its extremities. Howes and Swinnerton (1901) gave a critical account of the development of the vertebræ, described the presence of definite autogenous hypapophyses on both halves of a splitting vertebra and discussed the homology of the overarching chevrons on the first 4–5 caudal vertebræ, already noted by Dollo in 1883. Shauinsland (1906), on the basis of his

[1] Mr. Mahendra asks me to express his deep sense of gratitude to Prof. Gowland for the gift.

developmental studies, suggested that the spilt in the caudal vertebræ corresponds to embryonal segmentation. Woodland (1920) gave a sketchy account of the structure of its normal and regenerated tail for the sake of comparison with that of *Hemidactylus flaviviridis* Rüppel, which he studied minutely. Barbour and Stetson (1929) pointed out the resemblance of the scalation of the regenerated tail in *Sphenodon* to the scalation found in the jurassic Rhynchocephalian *Homœosaurus maximiliani*, and Wettstein (1931–32) summed up our knowledge of the caudal autotomy in this reptile.

III. Material and Methods

The specimen of *Sphenodon punctatus* studied by me was well preserved in formalin and it measured 10¼ inches from snout to vent. The tail measured 7·5 inches from the vent to its tip and included a terminal regenerated region two inches long. It was 1·4 in. wide at its base.

For the study of the musculature, the following three methods were employed:

(1) *Removal of the skin carefully from the underlying muscles*, in order to examine the superficial disposition of the musculature. This was done at three regions: the base of the tail, part of the normal autotomous region and part of the regenerated portion.

(2) *Breaking* of the normal part of the tail by lateral flexion. This was necessary for the study of autotomised surfaces and of the arrangement for the insertion of muscle processes of two adjoining autotomy segments.

(3) *Serial sections and reconstruction.* Transverse sections were cut, passing through the middle of the autotomy segments and between one autotomy segment and another. These were sketched twice the actual size on paraffin blocks of proportionate thickness and thus an accurate model was prepared to elucidate the disposition of the muscles and other parts of the tail.

The endoskeleton of the regenerated part was studied in transverse and longitudinal sections as well as by dissection.

IV. The Scalation

Since Boulenger (1888) suggested, on the basis of certain observations on *Gymnophthalmus* and *Ophisaurus*, that the aberrant scalation of the regenerated tail, in some cases at any rate, shows a reversion to a primitive ancestral type, the differences between the scalations of normal and regenerated tails have assumed a special significance. Barbour and Stetson's discovery (1929) of the scalation on the body and tail of a specimen of the

jurassic Rhynchocephalian ancestor *Homœosaurus* H. v. Mayer and its apparent similarity to that of the regenerated tail in *Sphenodon punctatus* seems to lend support to such a view.

As the caudal scalation of *Sphenodon* has not so far been described in detail, the following observations on the present specimen are recorded.

(i) *The Normal Tail.*—Situated mid-dorsally and extending backwards from the base of the tail, there is a longitudinal crest of enlarged tubercles (Plate IX, Fig. A), fifteen in number, the anterior ones being pointed and slightly recurved, whilst the posterior ones are shorter and blunt. These are the largest in size of all the caudal scales. The last of these tubercles lies immediately in front of the regenerated region, is the shortest in height and lacks a terminal point, there being a remarkable disparity between its size and the size of the tubercle immediately preceding it.

Only eight autotomy segments are distinct in front of the regenerated portion, each segment possessing one of the enlarged tubercles mid-dorsally and marked off clearly from the adjacent segments by an annular inter-segmental groove.[2] The absence of inter-segmental lines in the basal portion of the tail, corresponding to the seven anterior tubercles, is worth noting, as it apparently indicates a lack of autotomising ability in this region.

Laterally, the tail is studded with minute granular scales, subequal and irregular, and possesses two longitudinal series of major tubercles, and between them, another longitudinal series of minor tubercles. The latter coincides in its position with the lateral concave area, visible in the outlines of transverse sections, and corresponds with the point of attachment of the horizontal myoseptum to the body-wall.

The scales on the ventral aspect of the tail (Plate IX, Fig. B, and Text-fig.1) are flattened, regular, hexagonal and much enlarged. They are arranged in transverse rows of seven to twelve scales, the number decreasing posteriorly. In the region with distinct autotomy grooves, two transverse rows of ventral scales are seen to correspond to each autotomy segment, while in the region in front the arrangement is rather irregular. The autotomy grooves are apparently more marked than the ones separating the two transverse rows of the same autotomy segment. The first row in each segment consists mesially of 3 to 5 enlarged ventrals, while the second row possesses 7 to 8 such scales. Each of these rows is continued at the sides by a double row of smaller scales which pass over into the lateral scales of the tail.

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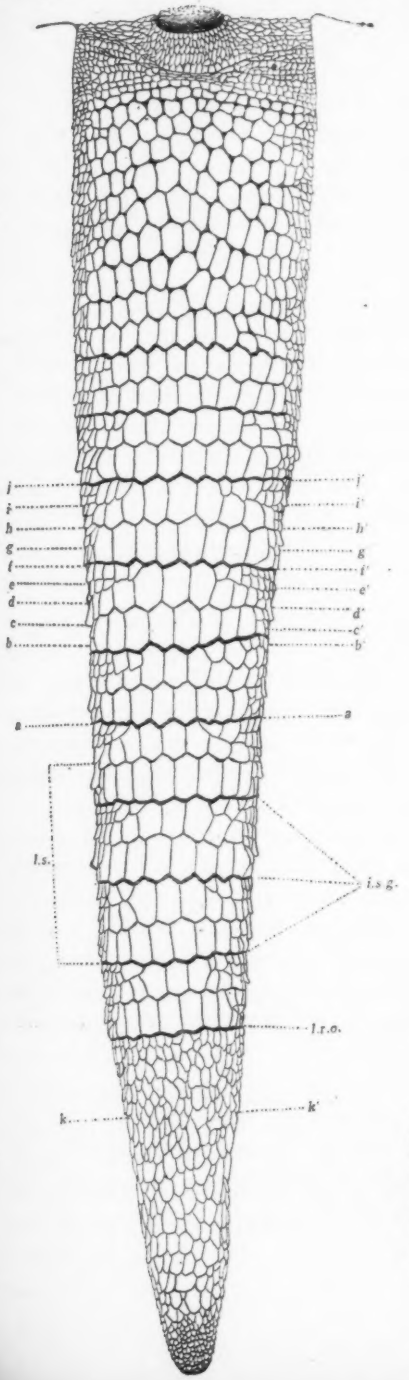
[2] The autotomy segments (unlike the vertebræ) correspond to the primary segmentation of the tail, as shown by Schauinsland and thus the lines separating them are really intersegmental in position.



TEXT-FIG. 1. The ventral scalation of the tail of *Sphenodon* (nat. size)

aa'—plane at which the tail was broken by lateral flexion; *bb'*, *cc'*, *dd'*, *ee'*, *ff'*, *gg'*, *hh'*, *ii'*, *jj'*—planes at which the serial sections were cut for reconstruction; *kk'*—plane at which transverse section of the regenerated part was prepared; *i.s.g.*—intersegmental grooves; *l.s.*—region at which the vertical longitudinal section was prepared; *l.r.o.*—line seaprating the original from the regenerated part of the tail.

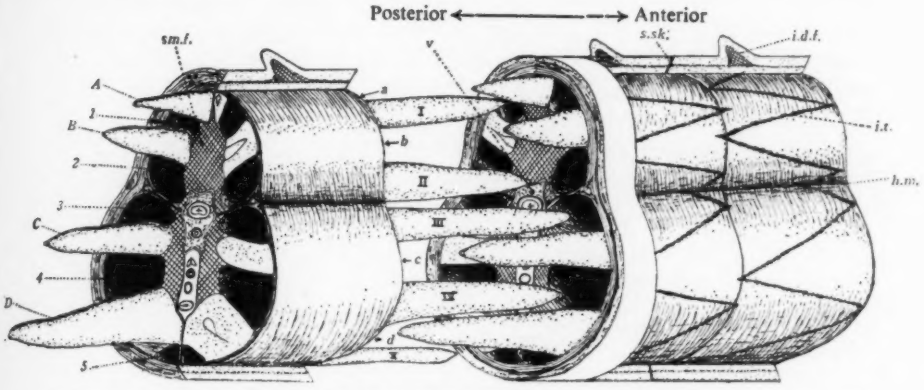
(ii) *The Regenerated Tail.*—Unlike the normal region of the tail, the regenerated portion (Plate IX, Text-fig. 1) shows no segmentation at all. The scalation is irregular. Dorsally, there is a double crest of tubercles, smaller, less conical and more obliquely set than the single ones in the normal tail. Laterally, the scales are definitely elongated, variable in size and more or less irregularly arranged. Ventrally, they are much smaller than the ones in the normal tail and are irregular in form and disposition, although flattened. The tip of the tail, bluntly rounded, is covered with extremely minute scales.

Regarding the scalation of the regenerated tail in *Sphenodon*, Wettstein (1932) remarks that it " consists of smooth polygonal, irregular and juxtaposed tubercles which are largest on the ventral side, become smaller towards the dorsal crest, but nowhere reach either the size and regular arrangement of the ventral scales, or the almost microscopic smallness of the lateral scales, of the original tail." In the specimen studied by me, the lateral scales of the original tail cannot, in any sense, be called microscopic (or almost microscopic), the great majority being about 1 mm. or more in width. They, however, differ from the lateral scales of the regenerated region in their form. They are oblong, while the regenerated laterals are distinctly elongated.

V. Autotomised Surfaces

The original tail of the specimen in question was broken at one of the autotomy segments (line *aa'* in Text-fig. 1) by lateral flexion. It was found that the breaking requires far more effort than that necessary in the case of *Hemidactylus*, *Lacerta*, *Mabuya*, or any other lizard with an autotomous tail. This relative difficulty has already been pointed out by Günther (1867, p. 606) and Hoffmann (1890, p. 476), although Wettstein (1932, p. 214) appears to be ignorant of it when he says, " Ob Sphenodon seinen Schwanz liechter oder schwerer abwirft als viele Lacertilier, ist nicht bekannt." The fact apparently has an evolutionary significance and seems to indicate that the process as seen in *Sphenodon* is more primitive and less specialized than that found in Lizards. As discovered by me (*vide infra*), the comparative difficulty in breaking off the tail of *Sphenodon* is correlated with certain important anatomical features.

In outline the broken faces of the autotomised tail (Text-fig. 2) are roughly triangular in appearance, the base of the triangle lying ventrally and the apex pointing upwards. The ventral surface is rounded, while each of the lateral surfaces has a concavity at about its middle due to a depression extending longitudinally along the entire tail.



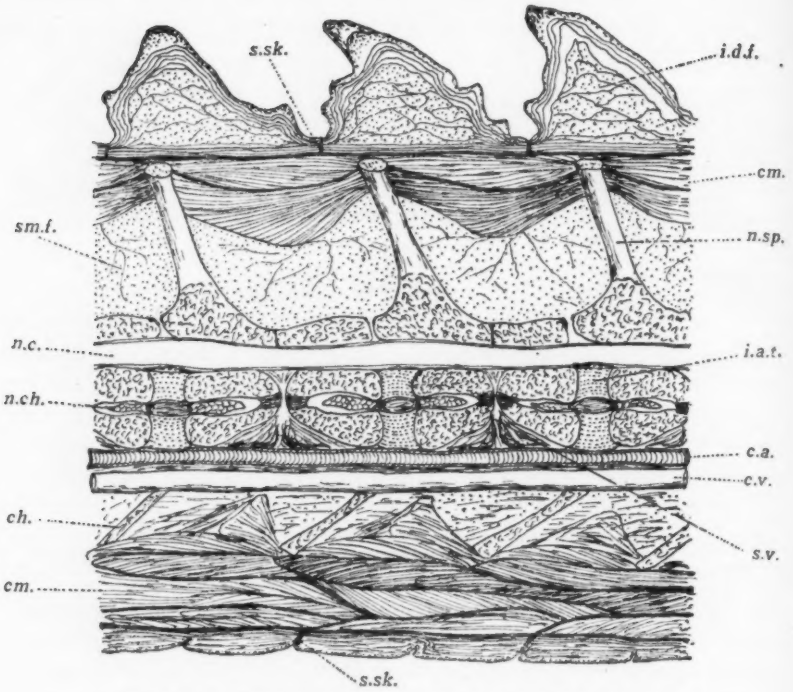
TEXT-FIG. 2. Diagram showing the autotomised surfaces in the broken tail of *Sphenodon*. The posterior processes on the right side of the last segment have been cut off and part of the skin of the first two segments removed

A, B, C, D—processes on the posterior surface, fitting into the cavities *a*, *b*, *c*, *d* on the surface opposite; I, II, III, IV, V—anterior processes fitting into the posterior cavities, 1, 2, 3, 4 and 5; *h.m.*—horizontal myoseptum; *i.d.f.*—intradermal fat deposit; *i.t.*—inscriptiones tendinæ; *sm.f.*—submuscular fat layer; *s. sk.*—split in the skin; *v.*—vertebra.

(i) *The Epithelium.*—The skin at the break shows no jagged appearance as should be expected if it were continuous from one segment to the other and would have been torn off by sheer force. On the other hand, it is fairly smooth and indicates the presence of definite pre-formed, annual zones of autotomy in the epithelium, which can clearly be seen in a longitudinal vertical section (Text-fig. 3).

The dermis, as in other Reptiles, is divisible into two main layers: a superficial *corium* and a deeper *subcutis*, the former being further subdivided into an outer, more loosely arranged *Stratum laxum corii* and a deeper compact-fibred *Stratum compactum corii* (Lange, 1931). The inner layers consist of numerous connective-tissue lamellæ lying more or less parallel to the exposed surface of the skin and enclosing a series of lacunæ here and there. The lacunæ are filled up with fat-cells and generally correspond in their location with the major elevations of the skin, particularly the dorsal and lateral tubercles.

Woodland (1920) has described, in the gecko *Hemidactylus flaviviridis* Rüppel, a prominent layer of fat-cells situated immediately under the skin. Such a subcutaneous fat-layer is altogether absent in *Sphenodon*, the fat-lacunæ described above being distinctly intra-cutaneous rather than subcutaneous. The absence of this layer in *Sphenodon* has not been previously noted.



TEXT-FIG. 3. Longitudinal vertical section through the original tail

c.a.—Caudal artery; *ch.*—chevron bone; *c.m.*—caudal muscles; *c.v.*—caudal vein; *i.a.t.*—interarticular tissue; *n.c.*—neural canal; *n.ch.*—notochord; *n.sp.*—neural spine; *s.v.*—split in the vertebra. (Other abbreviations as in the previous Fig.)

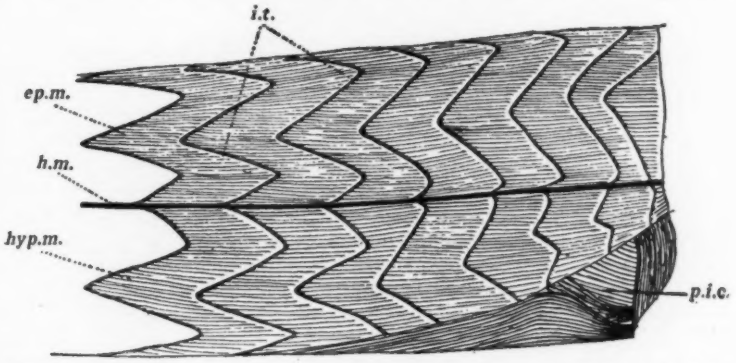
(ii) *The Caudal Musculature*.—The innermost layer of dermal connective tissue is produced mesially to form a horizontal *myoseptum*, dividing the caudal musculature into a dorsal (*epiaxonic*) and a ventral (*hypaxonic*) set. The outer end of the septum underlies the longitudinal depression visible externally on the lateral surface, while the inner end is connected to the vertebral column.

Beneath the dermis lie the caudal muscles, which are produced into processes both on the posterior face of the tail-stump and the anterior face of the separated caudal region. The processes of one autotomy segment dove-tail into those of the adjoining one and there are definite cavities to accommodate them.

The presence of the horizontal myoseptum makes it possible to distinguish the processes and cavities situated dorsal to it from those lying ventral to it. I shall call the dorsal ones *epiaxonic* and the ventral ones *hypaxonic*.

On the posterior face of the tail-stump (Text-fig. 2) there are on each side two epiaxonic (A, B) and two hypaxonic (C, D) muscle processes, and two epiaxonic (1, 2) and three hypaxonic cavities (3, 4, 5). The processes alternate with the cavities except at three places: (*a*) mid-dorsally, where the members of the first pair of processes are adjacent, (*b*) mid-ventrally, where the members of the ventralmost pair of cavities lie close to each other, and (*c*) near the horizontal myoseptum, where an epiaxonic and a hypaxonic cavity lie separated by it.

On the anterior face of the separated tail-piece, there are on each side two epiaxonic (I, II) and three hypaxonic (III, IV, V) processes, and two epiaxonic (*a*, *b*) and two hypaxonic (*c*, *d*) cavities. The arrangement of these is the reverse of those found on the posterior face of the tail-stump.



TEXT-FIG. 4. Superficial appearance of the muscles at the base of the tail after removal of the skin (Right side)

ep. m.—epiaxonic muscles; *hyp. m.*—hypaxonic muscles; *p. i. c.*—*Pars ischio-caudalis* of *M. ilio-ischio-caudalis*. (Other abbreviations as in previous Figs.)

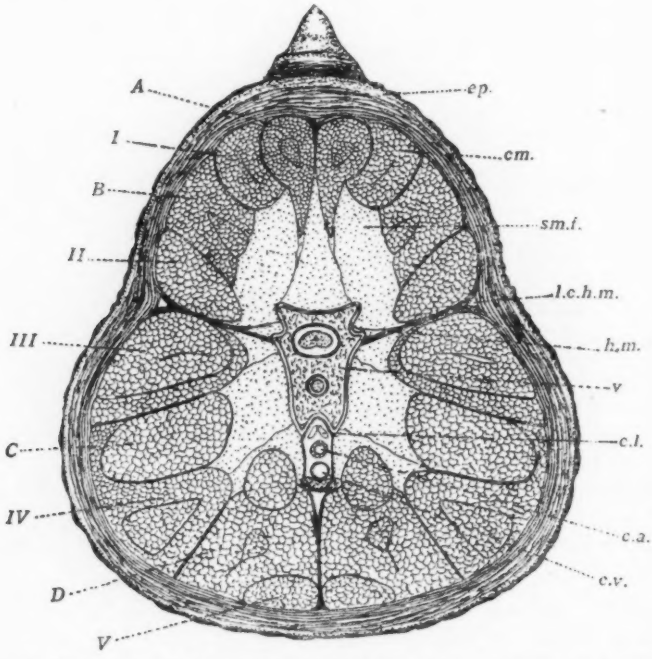
It is surprising to note that none of the previous workers on caudal autotomy mention any projecting processes on the anterior aspect of the broken-off piece of the tail either in the Lizards studied by them or in *Sphenodon*. These processes in *Sphenodon* are very conspicuous and are lodged in considerably deep cavities on the posterior face of the tail, which have also escaped previous observation.

Further, the distinction between the *epiaxonic* and *hypaxonic* muscle processes and cavities, which is extremely important for tracing the homology of these parts, has not been made before.

The arrangement and number of the muscle processes and cavities in *Sphenodon*, as described by me, differ from those in *Hemidactylus* (Woodland, 1920) in the following important details, not recorded before:

(1) The posterior face of the tail-stump has five pairs of cavities, while there are only four such pairs in *Hemidactylus*.

(2) There are four pairs of large processes projecting considerably forwards from the posterior face of the tail-stump, while there are none described in *Hemidactylus* by Woodland. The dorsalmost and the ventralmost of these processes in *Sphenodon* are probably represented by "two pairs of small tapering muscle extremities" in *Hemidactylus*, one in the mid-dorsal line and the other in the mid-ventral.



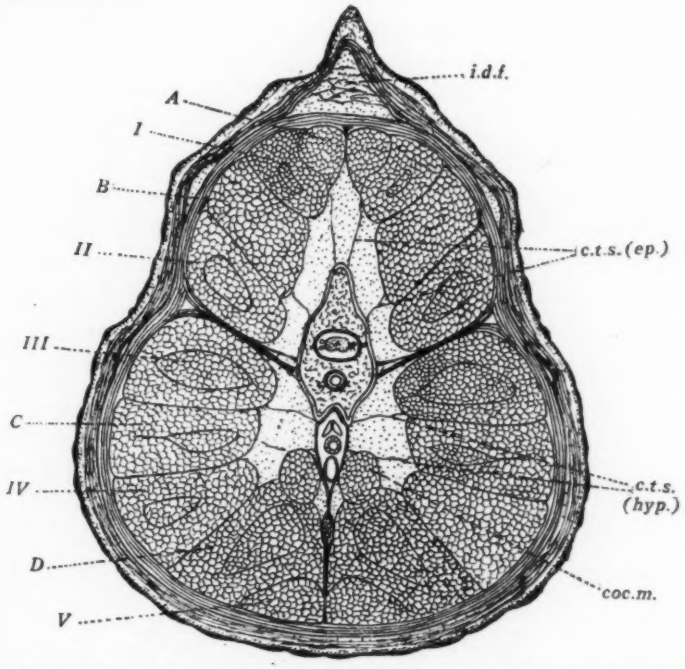
TEXT-FIG. 5. Transverse section passing through the plane *jj'*, shown in Text-fig. 1

c. l.—caudal lymphatic trunks; *ep.*—epithelium; *l.c.h.m.*—lateral longitudinal channel at the outer border of the horizontal myoseptum. (Other abbreviations as in previous Figs.).

(3) The muscle processes in *Sphenodon* are arranged in two lateral series each consisting of four on the posterior face of the tail-stump and five on the anterior face of the separated piece. In appearance, the arrangement of the muscle processes in *Sphenodon* is more reminiscent of the condition described by Sibtain (1938) in *Mabuya dissimilis*, which also possesses a laterally compressed tail, than that found by Woodland (1920) in *Hemidactylus*, which has a dorso-ventrally depressed tail.

(4) There are four pairs of deep cavities on the anterior face of the broken-off tail piece, while there are none described in *Hemidactylus*.

(iii) *The Sub-muscular Fat Layer*.—Underneath the caudal muscles and outside the vertebral column lies a well developed *fatty tissue* similar to the one found in *Hemidactylus* (Woodland, 1920), *Lacerta* (White, 1925) and *Mabuya* (Sibtain, 1938).



TEXT-FIG. 6. Transverse section passing through the plane *hh'*, shown in Text-fig. 1

coc. m.—coccygeal muscles; *c. t. s.* (*ep.*)—connective tissue septa dividing the epiaxonic submuscular fat layer; *c. t. s.* (*hyp.*)—connective tissue septa dividing the hypaxonic submuscular fat layer. (Other abbreviations as in previous Figs.)

This layer in *Hemidactylus*, according to Woodland (1920), is "segmented by lines or rather planes of cleavage" continuous with those of the skin and the subcutaneous fat layer. In *Sphenodon*, however, I have not found in this layer any *transverse segmentation* by cleavage planes.

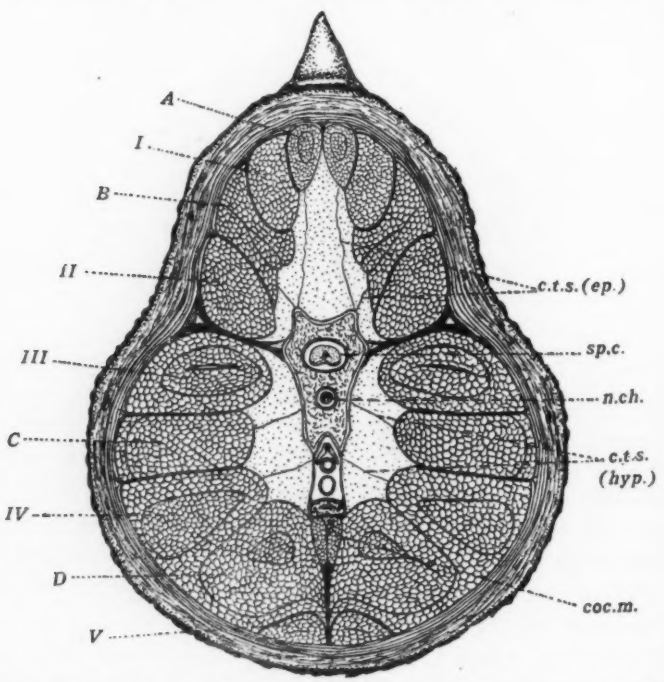
In autotomised surfaces, more distinctly in transverse sections (Text-fig. 6), the fat layer is seen to be traversed by four pairs of connective-tissue septa, two situated dorsal to the horizontal myoseptum and two ventral to it. The septa are radial in position but extend rather obliquely in their

longitudinal course so that some of them are visible even in longitudinal vertical sections.

The division of the fatty layer into four fat-bands as described in *Hemidactylus* (Woodland, 1920) is lacking in *Sphenodon*, but the radial septa divide this layer into eleven sub-divisions: five epiaxonic, and six hypaxonic (Text-fig. 6). Of the epiaxonic sub-divisions of the fatty layer, a single one lies mid-dorsally above the neural spines and the inter-spinous portions of the vertebræ; one pair above the zygapophyses and on either side of the neural spine in the interzygapophyseal region; and another pair on either side of the base of the neural arch. Of the hypaxonic sub-divisions, the upper one is situated on either side of the centra, while the lower two lie on either side of the hæmal canal.

The fatty tissue serves to fill up the spaces or lacunæ lying between the bands of caudal muscles on the one hand and the vertebræ on the other.

(iv) *The Caudal Vertebræ and their contents.*—Internal to the sub-muscular fatty tissue is the vertebral column of the tail, enclosing the caudal



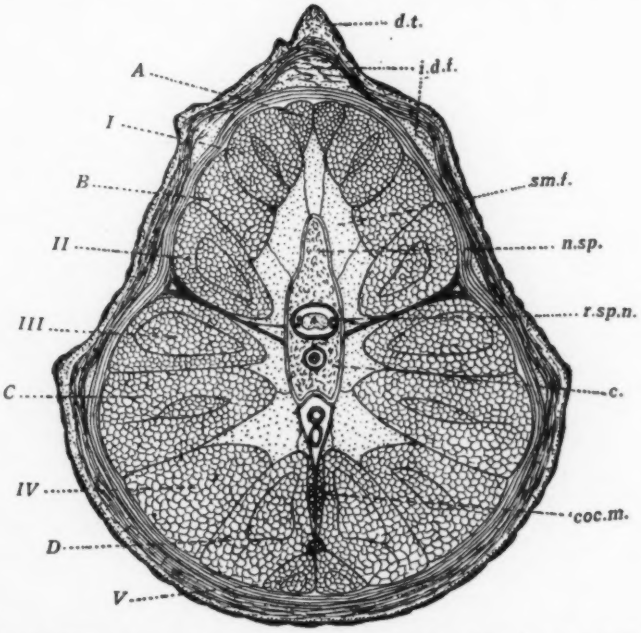
TEXT-FIG. 7. Transverse section passing through the plane *ff'*, shown in Text-fig. 1 *sp. c.*—spinal cord. (Other abbreviations as in previous Figs.)

portion of the spinal cord inside its neural space, the remains of the notochord inside the centra, and the caudal vessels in the hæmal space. The split in the caudal vertebra, as already known, passes approximately through the middle and divides it into two sub-equal halves, the posterior one bearing both transverse processes and the neural spine (Günther, 1867; Gadow, 1896; Howes and Swinnerton, 1901).

The serial transverse sections (Text-figs. 6 and 8) of the tail show clearly that the spinal nerves take their origin at the middle of an autotomy segment. Both the dorsal and the ventral roots are visible on either side of the spinal cord within the neural space.

(v) *The Caudal Vessels.*—Immediately underneath the centra is a *caudal canal*, enclosed partly by the chevron bones and mostly by a tough sheath of connective tissue. In the dorsalmost part of this canal, two longitudinal, *lymphatic trunks* (Text-figs. 5–8) extend all along the length of the tail. Below them is the *caudal artery*, and below the caudal artery, the *caudal vein*.

The calibre of the caudal artery shows no noticeable difference in the various sections, nor does its wall present any thickening corresponding to the sphincters in the caudal artery in *Hemidactylus* (Woodland, 1920).



TEXT-FIG. 8.—Transverse section passing through the plane *dI'*, shown in Text-fig. 1 *r. sp. n.*—roots of spinal nerves. (Other abbreviations as in previous Figs.)

Besides the lymphatic trunks mentioned above, there are two lateral channels lying along the outer border of the horizontal myoseptum, where it meets the dermis. These probably are also lymphatic vessels.

VI. The Caudal Musculature

(i) *The Normal Tail.*—The horizontal myoseptum divides the musculature of the tail into a dorsal or *epiaxonic* and a ventral or *hypaxonic* set.

The epiaxonic set, called the *Caudæ dorsalis* by Byerly (1925), appears to correspond to the two longitudinal muscle-systems of the trunk, the *Musculus longissimus*[3] and the *transverso-spinalis* (Nishi, 1937), which have almost completely formed a single muscle here.

The hypaxonic set is formed, along its greater length, by the *Musculus ilio-ischio-caudalis*, which overlies the *coccygeal musculature* at the base of the tail (Wettstein, 1932). A part of the *ilio-ischio-caudalis* at the cranial part of the tail can be distinguished from the rest as an unsegmented muscle, the *Pars ischio-caudalis*.

Superficially, both the epiaxonic and hypaxonic caudal muscles (Text-fig. 4) are segmented by a series of zigzag lines, the *Inscriptiones tendinæ*, the arrangement of which is described by Wettstein as follows:

> " This muscle is traversed by metameric myosepta which form *inscriptions* on its superficial surface, having the form of a Σ directed anteriorly. Both the caudalwards open angles, as well as the cranialwards open middle angle of this Σ form fleshy cones penetrating obliquely into the depth of the muscle. Thus each metamere of this muscle consists, on each side of the dorsal median line, of three alternating muscle-cones, each of which is covered with a delicate *fascia*. The cones lying one behind the other in a longitudinal series are inserted into each other in the form of paper bags."

As shown by me in the previous section, each metamere of the dorsal caudal muscle projects out, not only anteriorly but also posteriorly, into two processes of considerable length and thus possesses not three alternating cones as described by Wettstein, but actually four. The anteriorly directed pair of cones belonging to one metamere alternates in its position, with the posteriorly-directed pair of the metamere in front, and thereby two successive metameres can dovetail into each other. The muscle-cone missed by Wettstein is evidently the dorsal one on the posterior face of the metameres.

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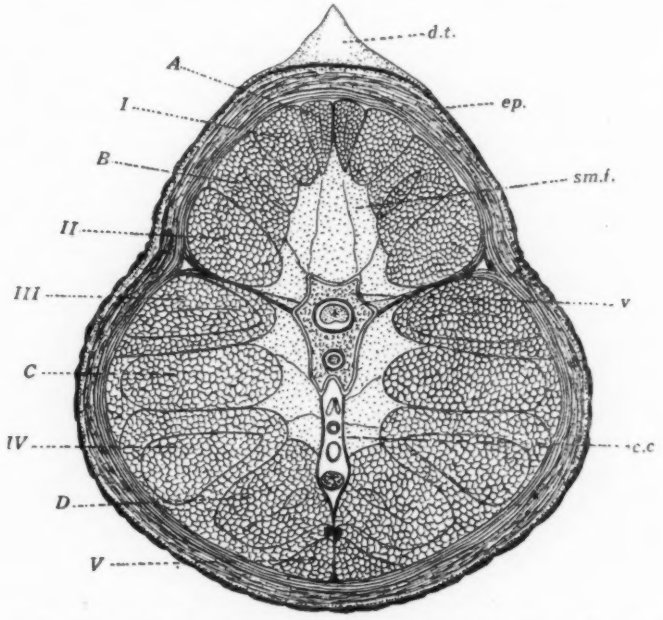
[3] This probably also includes the Iliocostalis elements (Nishi, 1937, p. 388).

In order to study the relative sizes and the precise mode of fitting up of the muscle processes of the caudal musculature, both epiaxonic and hypaxonic, a series of nine hand-cut sections were prepared, passing through the planes *bb'*, *cc'*, *dd'*, *ee'*, *ff'*, *gg'*, *hh'*, *ii'* and *jj'*, as indicated in Text-fig. 1. Of these the sections *bb'*, *ff'* and *jj'* passed through the planes of autotomy; while the sections *dd'* and *hh'* were exactly midway between two such successive planes. The sections *cc'*, *ee'*, *gg'* and *ii'* passed between the planes of autotomy and the middle of the autotomy segments.

A study of these serial sections by the reconstruction method brings out several interesting details, which are mentioned below:



TEXT-FIG. 9. Transverse section passing through the plane *bb'*, shown in Text-fig. 1

c.c.—caudal canal containing blood vessels, etc.; *d. t.*—dorsal tubercle. (Other abbreviations as in previous Figs.)

(1) The posteriorly-directed epiaxonic processes (A and B in Text-figs. 6 and 8) do not show any inserted process inside them at the level of the middle of each autotomy segment, while at the plane of autotomy (Text-figs. 5, 7 and 9) they do have such a process inside. This shows that these processes do not extend farther than the middle of the successive autotomy segment.

(2) The anteriorly-directed epiaxonic processes are just the reverse of the posteriorly-directed ones in their arrangement. They (I and II) are solid at the planes of autotomy (Text-figs. 5 and 7), but possess an inserted process at the level of the middle of each autotomy segment (Text-figs. 6 and 8). Thus they extend farther than the middle of the preceding autotomy segment, but end a little behind the preceding autotomy plane.

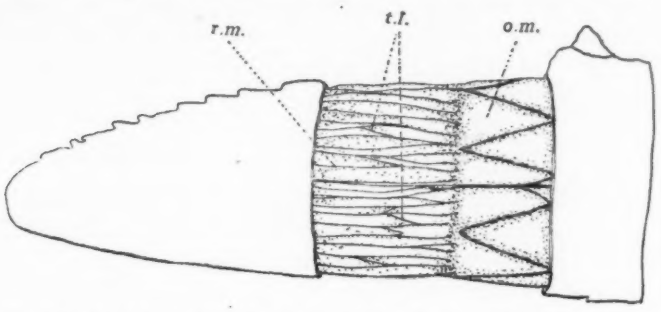
(3) The anteriorly-directed, ventralmost pair (V) of hypaxonic processes resembles the anteriorly-directed epiaxonic ones in size and arrangement. These are solid at the planes of autotomy (Text-figs. 5, 7 and 9), but possess an inserted piece at the level of the middle of the autotomy segments (Text-figs. 6 and 8).

(4) The dorsal one (C) of the two posteriorly-directed hypaxonic pairs although extending in the opposite direction, is similar in extent and arrangement to the ventralmost hypaxonic pair, mentioned in the foregoing paragraph. It is solid at the autotomy plane, but contains an inserted process at the middle of the autotomy segment.

(5) The remaining three pairs of hypaxonic processes (III, IV and D) differ from all the others in length, extending apparently farther than the limits of the adjacent segment. Of these, two (III and IV) are directed anteriorly, while the third (D) runs posteriorwards.

(6) The *coccygeal musculature*, which is visible as a pair of two muscles lying one on each side of the hæmal arches of the anterior caudal vertebræ (Text-figs. 3 and 4), when traced backwards, shows two features worth observing (Text-fig. 6). In the first place, the two muscles forming it approach each other more and more until they lie approximated in the median line (Text-figs. 7 and 8). Secondly, they shift downwards to assume a position below the hæmal space and lie wedged in between the members of the fourth pair (D) of hypaxonic muscle-processes.

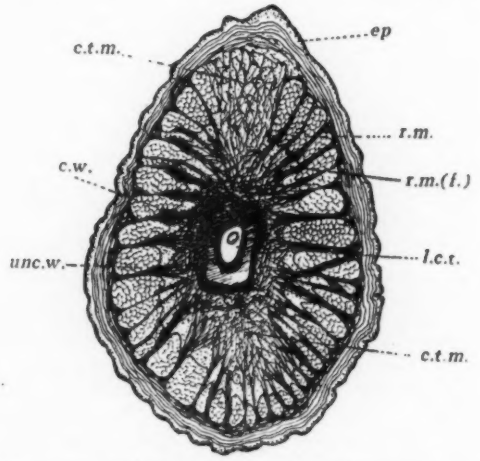
(ii) *The Regenerated Tail.*—Regarding the musculature of the regenerated tail in *Sphenodon*, Wettstein (1932) remarked that the muscles form, around the cartilage, an *unsegmented* covering which is separated from it by connective tissue. Woodland (1920) found that the muscles in the regenerated tail of *Hemidactylus flaviviridis* run in a straight line along its entire length and are devoid of transverse segmentation (*cf.* Text-fig. 4 A of Woodland, 1920). White (1925) observed distinct transverse segmentation in the musculature of the regenerated tails in *Lacerta vivipara*, while Sibtain (1938), although reporting an apparent, unsegmented appearance of de-skinned regenerated muscles, established (by breaking) the presence of a dove-tailing arrangement even in them in *Mabuya dissimilis*.



TEXT-FIG. 10. Superficial appearance of the musculature (after removal of skin) at the place of junction of the original and regenerated parts of the tail.

o. m.—muscles of the original part of the tail; *r. m.*—regenerated muscles; *t. l.*—transverse lines dividing regenerated muscles.

In the specimen of *Sphenodon* studied by me, the removal of skin from the regenerated region showed that the muscles (Text-fig. 10) do not form continuous longitudinal bands running all along up to the tip, but show irregular transverse divisions by oblique lines. They are extremely small in comparison to the muscles of the original tail, are arranged in an outer row just underneath the dermis and are fairly numerous (over forty).



TEXT-FIG. 11. Transverse section passing through the regenerated part of the tail (Plane *kk'*, in TEXT-FIG. 1)

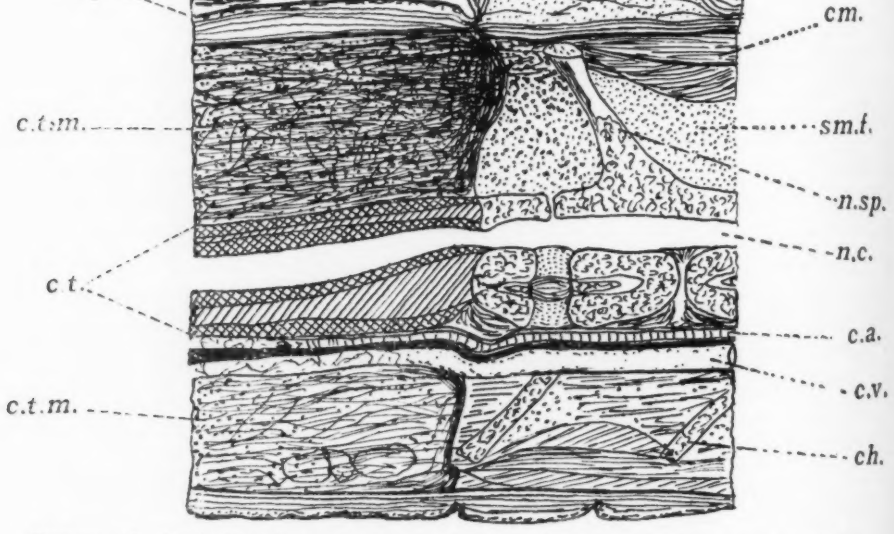
c.t.m.—connective-tissue meshwork; *c. w.*—calcified part of the wall of cartilaginous tube; *ep.*—skin; *l. c. t.*—lumen of the cartilaginous tube; *r. m.*—regenerated muscles; *r. m. (f).*—fatty inner ends of regenerated muscles; *unc. w.*—uncalcified portion of the wall of the cartilaginous tube.

Transverse sections of the regenerated tail (Text-fig. 11) establish clearly that the muscles in this region lie side by side, separated by radial connective-tissue septa, and are devoid of any inserted pieces as described in the case of the original tail. Mid-dorsally, the row of muscles is interrupted.

VII. The Endoskeleton and other Parts of the Regenerated Tail

(*i*) *The Epithelium.*—The skin in the regenerated part differs from that in the original in three important ways: (*a*) it is distinctly thinner; (*b*) it does not show intra-dermal fat deposits as found in the original tail; and (*c*) it is continuous from the place where regeneration started to its tip, having no splits corresponding to those found at the autotomy planes in the original tail.

(*ii*) *The Muscles and the Underlying Tissue.*—The musculature of the regenerated tail has already been described in Section VI, but we may note here that in transverse sections (Text-fig. 11) each muscle bundle shows fatty degeneration towards its inner end and that there is no distinct continuation of the horizontal myoseptum. The regenerated muscles cannot



TEXT-FIG. 12. Vertical longitudinal section passing through the place of junction between the original and regenerated parts of the tail

c.t.—Cartilaginous tube. (Other abbreviations as in Text-figs. 3 and 11.)

be regarded as functional as they lack insertion on the axial skeleton. They are separated from each other by radial connective tissue septa, which extend inwards towards the cartilaginous tube (*vide infra*) and unlike those of Hemidactylus (Woodland, 1920), ramify to form a meshwork lying underneath the musculature. The meshes are filled up with fat deposits.

The caudal vessels extend longitudinally into the regenerated part ventral to the cartilaginous tube, but the transverse sections show that they are situated neither symmetrically in the median line, nor one below the other in close juxtaposition.

(*iii*) *The Cartilaginous Tube.*—The cartilaginous tube, which forms the axis of the regenerated region, forms an extension of the vertebral canal (Text-fig. 12) and is irregularly shaped in successive transverse sections. As in the regenerated tail of *Hemidactylus flaviviridis* (Woodland, 1920) its wall is calcified on its peripheral and inner surfaces and uncalcified between the two. The uncalcified portion, however, does not present a continuous annular appearance in many of the transverse sections, being divided at one or two places by a union of the peripheral calcified portion with the inner calcified one. Such particularly is the case along the greater anterior region of the regenerated part, where the latter is joined to the original tail-stump.

VIII. Conclusion

In a previous section, the relative difficulty experienced in breaking the tail of *Sphenodon* as compared to that in fragile-tailed Lizards was particularly noted. This is due to the fact that the tail of *Sphenodon* is less specialised for autotomy, a conclusion which is fully substantiated by the numerous morphological features, already described. The presence of well developed muscle-pegs on both the faces of each autotomy segment and their mode of insertion, the absence of cleavage lines in the submuscular fatty tissue, the absence of the subcutaneous fat bands, the absence of sphincters in the caudal artery (noted by Woodland, 1920), and the proportionately less, fatty degeneration shown by dermal, muscular and connective tissues: all these are features which can be interpreted only in this way.

IX. Summary

The author has given a detailed description of the anatomical features both of the original and the regenerated tail in *Sphenodon punctatus*. The following are the more important features dealt with by him:

(1) A complete account of the scalation both of the original and regenerated regions is given.

(2) The greater difficulty experienced in breaking the tail of *Sphenodon* is noted, as compared to that in fragile-tailed lizards. It has been correlated with the morphological details of the autotomy segments.

(3) The skin shows pre-formed lines of cleavage.

(4) The subcutaneous fat-layer is absent, but there are intra-dermal lacunæ filled up with fat deposits, and corresponding generally with the major elevations of the surface in their positions.

(5) The position of the horizontal myoseptum is described and its importance indicated in dividing the caudal musculature into homologically different sets.

(6) The difference in the number and arrangement of the muscle processes from that found in *Hemidactylus* is pointed out. Amongst the main characters, not noted by previous workers in this connection, are: (*a*) the presence of projecting processes on the anterior aspect of the broken-off piece; (*b*) the presence of five (not four) pairs of cavities on the posterior face of the tail-stump; (*c*) the presence of four pairs of large processes projecting from the posterior face of the tail-stump; and (*d*) the presence of four pairs of deep cavities on the anterior face of the broken-off tail piece.

(7) The sub-muscular fat layer is not segmented by lines of cleavage. The radial connective-tissue septa traversing this layer and the eleven subdivisions consequently resulting have been described in detail.

(8) The caudal artery, vein and lymphatic trunks have been described.

(9) The caudal musculature has been carefully studied by the reconstruction method and it has been shown that the dorsal pair of muscle-cones on the posterior face of the metameres was missed by previous workers.

(10) The insertion of the individual muscle processes has been precisely described. No account of these by any previous worker has so far been published.

(11) The skin of the regenerated part is distinctly thinner than that in the original, has no fat-deposits and is devoid of transverse lines of cleavage.

(12) The muscles of the regenerated tail show transverse segmentation, have no inserted pieces inside them, and are arranged side by side, separated by radial connective-tissue septa.

(13) The muscle bundles in the regenerated tail show fatty degeneration towards their inner ends.

(14) Internal to the row of muscles, the regenerated tail possesses a meshwork formed by the ramification of the connective-tissue septa, and the lacunæ inside the meshes are filled with fat-deposits.

(15) The caudal artery and vein, although continued into the regenerated tail, lie wide apart from each other, being not situated in the mid-longitudinal line below the cartilaginous tube.

(16) The difference in the structure of the wall of the cartilaginous tube from that described in *Hemidactylus* has been noted.

X. Acknowledgments

The present work was carried out, during the course of my vacations, under the direction of Mr. Beni Charan Mahendra at the Department of Zoology, St. John's College, Agra, and I am very grateful to the authorities of the College for permission to work in the Laboratory. I wish to express my deep sense of gratitude to Mr. Mahendra not only for the invaluable specimen of *Sphenodon* he gave me for study but also for his continual help and guidance. I am also indebted to Mr. D. Raja Ram, Principal, St. Andrew's College, Gorakhpur, for the encouragement he has given me.

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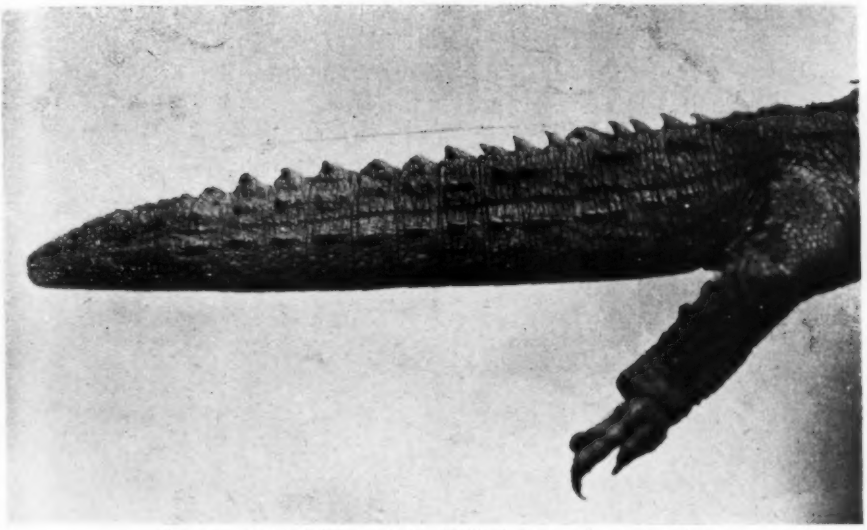

FIG. A. Lateral View of the Tail of *Sphenodon punctatus*

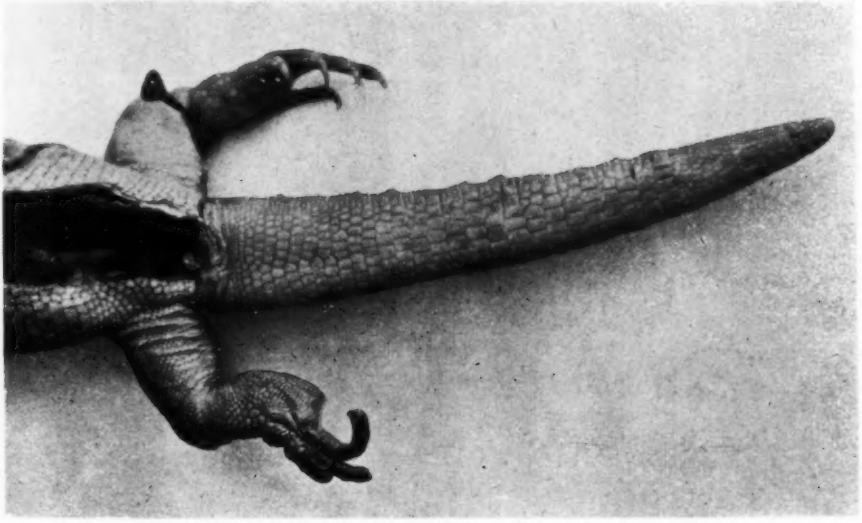

FIG. B. Ventral View of the Tail of *Sphenodon punctatus*

ON A NEW SPECIES OF *PRAEGERIA* OCCURRING IN THE SANDY BEACH, MADRAS*

By K. H. Alikunhi, B.Sc.

(*From the University Zoological Research Laboratory, Madras*)

Received February 14, 1941

(Communicated by Prof. R. Gopala Aiyar)

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Historical, Material and Methods

The genus *Praegeria* belonging to the family Pisionidæ was first constituted by Southern in 1914 for the reception of a small polychæte obtained from Clew Bay during Clare Island Survey and the genotype *P. remota* has been briefly described by him. Later in 1932 Smith has recorded the abundant occurrence of this species in the infauna of the shell-gravel deposits of the Eddystone Grounds near Plymouth. The present form is the next addition to be made to the genus and is the first record of *Praegeria* from India. A single mature male specimen of the species under consideration was also

* Paper read at the 27th Session of the Indian Science Congress held at Madras in January, 1940.

obtained from the sandy beach at Pathinettarayālom on the West Coast (Malabar) of India.

The worms live in large numbers in coarse sand from half to low water level of the intertidal region of the Madras Beach. Like most of the intertidal animals the range of distribution of these worms in the tidal zone is limited and examination of samples of sand from low to high water level reveals a steady decrease in their number until they are completely absent from about 2/3 tide level upwards. With regard to the habitat of *P. remota* Southern says, ' It is a small species living on a bottom of sand and shells or gravel and would escape capture by the dredge unless special precautions are taken '. As has been already mentioned the Madras form also prefers a coarse substratum for habitat.

The time of low tide is the most favourable for collecting these minute worms. When a quantity of sand from the particular zone in which the worms are found is taken in a glass trough and vigorously shaken with sea water they come up and swim in the supernatant water. This water is then quickly decanted into a glass dish from which the worms could be easily pipetted out. Collections at the spot are made by directly pipetting the worms from the disturbed sand (and water) even though this method is more tiresome. When disturbed the worms roll themselves into minute balls which are practically indistinguishable amongst the sand grains, and then firmly attach themselves to the bottom by the posterior end. It may be mentioned that the worms can be obtained practically throughout the year. However, during November and December when the salinity of the shore water is considerably lowered and undergoes sudden variation due to the N. E. Monsoon and the influx of fresh water from the Cooum and the Adyar, most of the worms perish and only few of them manage to survive.

The following account is based upon observations on living worms, whole mounts and serial sections. Fixation in Bouin's fluid gave good results. Double embedding in cedukol was also found to be very useful, especially for longitudinal sections. Iron hæmatoxylin and Delafield's hæmatoxylin were used for staining.

In the course of the present investigation two more species of *Praegeria* —*P. remota* and a second new species, *P. complexa*,—have been discovered and have been the subject of detailed study, the results of which will form the subject-matter for separate papers. Knowing, therefore, the anatomical features of three species of the genus of this little known family (Pisionidæ) a comparison of the forms has been made possible.

External Characters

The worms are very small and slender and measure about 3 to 10 mm. in length. Segmentation is distinct and the number of segments varies from 20 to 50 in the mature individuals. The male is invariably smaller than the female. The fully ripe males possess a small white area in one of the middle segments of the body, representing the position of the sperm-sacs, while in the ripe female the second half of the body is greenish in colour due to the accumulation of ova. By these characters alone the sexes can be distinguished with the unaided eye.

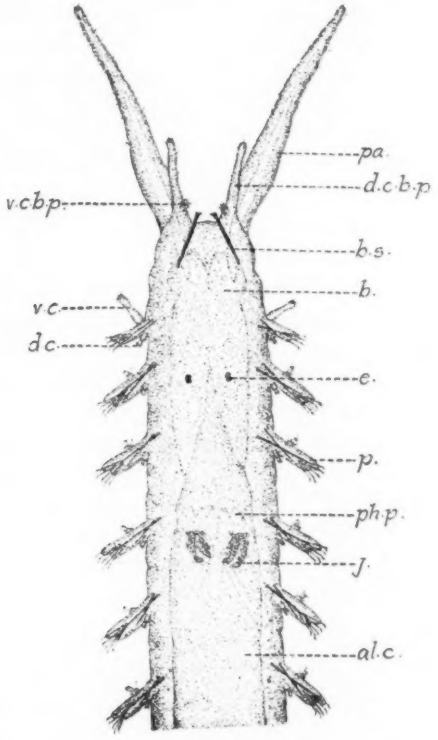


FIG. 1

In the general structure and build of the body the present form (Fig. 1 and Photomicrograph 1) resembles *P. remota*. The body which gradually tapers to the tail, is often flesh coloured and as mentioned above, this colouration makes it difficult to distinguish them when amongst the

sand grains. The segments are broader than long but at the posterior extremity a few of the segments are longer. The parapodia are all well developed and conspicuous. The head is highly reduced and fused with the anterior segments such that there is no marked differentiation of the former from the trunk. The brain is anteriorly situated and is in the form of two elongated lobes fused in front and stretching behind into the posterior half of the third setigerous segment. At the level of the second setigerous segment and placed on the lobes of the brain in direct communication with them is a pair of dark eyes.

The bases of the buccal parapodia are highly swollen and are fused in front of the head, the fusion being marked by well defined deep grooves. The groove on the ventral side is deeper and extends upto very near the mouth. The parapodial base is supported by a small spine, usually about 66 microns long. It is slightly swollen in the middle and is expanded at the tip which is smooth and devoid of any teeth or serrations. Each of these spines is placed in a slanting position, pointing forwards and towards the median line, so that the tips of the two often touch each other. In contrast to *P. remota* these spines are small and more anteriorly situated, there being a considerable space between their inner ends and the base of the first setigerous foot. The swollen base of the buccal parapodium is prolonged dorsally into a slender tapering cirrus, usually measuring about 115 microns in length. Beneath the base of this is a minute globular cirrus with a few palpocils at the tip. These two probably represent the dorsal and ventral cirri of the buccal parapodium. To the ventral surface of the buccal parapodia are attached the bases of a pair of large tapering cirri measuring about 350 microns in length. These are the palps. Towards the base of each palp is a conspicuous collar-like tissue or sheath from which it appears to pass to the ventral surface of the head above the mouth. The palps and cirri have a ringed or jointed appearance and are provided with palpocils at every constriction.

Parapodium.—The parapodia are all uniramous. The first setigerous foot is slightly smaller than the succeeding ones and has the ventral cirrus slightly elongated, measuring about 30 to 40 microns in length. In *P. remota* the ventral cirri of the first pair of parapodia are considerably elongated so that when directed forwards they reach beyond the base of the palps; but in the present form these cirri when directed forwards reach only half way to the base of the palps.

All the parapodia are almost similar in structure. The tenth foot may be described here (Fig. 2). The main setigerous lobe is elongated and deeply

bifid at the tip, having two blunt processes or papil'æ. The ventral one of these papil'æ is more pointed and projects beyond the dorsal one. The dorsal and ventral cirri are globular structures attached by narrow bases and with minute terminal papil'æ carrying long palpocils. The setigerous

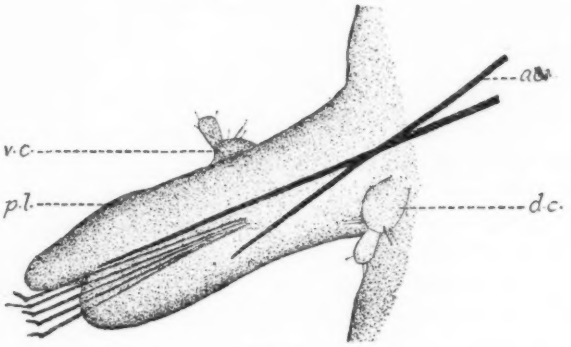


FIG. 2

support of the parapodium consists of two acicula and five setæ. Of the two acicula which are simple spines, one is smaller than the other and is situated in the dorsal part of the parapodial lobe. Among the five setæ one is simple while the rest are compound. This simple seta, which is present in all the feet, is thicker than the compound ones and has an expanded and bevelled tip provided with a row of minute teeth (Fig. 3 *a*). Of the compound setæ the one situated nearest to the simple seta differs from the rest in having

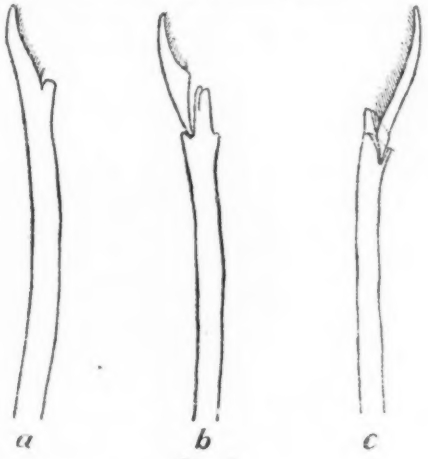


FIG. 3

a longer terminal blade and in being more slender (Fig. 3 *b*). In the structure and articulation of this longer terminal blade also this seta differs from the other compound setæ. The compound setæ are deeply bifid at the tip (Fig. 3 *c*) and carry lateral projections to one of which the terminal piece is articulated. The terminal blades of each of the setæ are provided with a row of slender teeth.

The setigerous support of the first foot consists of only four setæ, one compound seta being absent. Also in the last four or five pairs of parapodia the setæ are reduced to four in number.

Southern mentions the occurrence of, and figures, a second simple seta in the posterior segments of *P. remota* beginning from the 10th parapodium. In the present form this seta is absent. Again the compound seta with the longer terminal blade, present in every foot of the Madras species, is also seen in other specimens of *Praegeria* which I am inclined to regard as *P. remota* though the presence of these long bladed setæ are not mentioned in Southern's description of *P. remota*.

In the young ones of the Madras form, *i.e.*, in worms with 10 to 12 segments only, each foot is provided with only 4 setæ—the compound seta with the longer terminal blade being absent from every foot.

The Caudal Glands.—The anal segment is very peculiar and forms one of the important distinguishing features of the species (Fig. 4). On either side of the median line of this segment is a conspicuous group of caudal

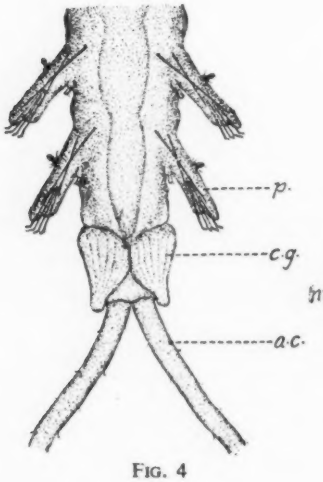


FIG. 4

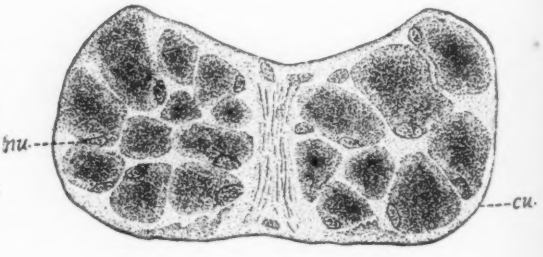


FIG. 5

glands. In living worms, under the microscope, these glands have a transparent, refractile appearance and the two groups together slightly exceed the width of the preceding segment. Each group is a compact structure, broad anteriorly, and tapering to the hind end (Photomicrograph 2) and is formed of 6 to 10 elongated gland cells having separate external openings. In sections (Fig. 5) it is seen that these glands are held together by a common outer covering. When a living worm is pressed under the coverglass and examined, the glands are seen to contain quite a large number of closely packed thread-like structures. The ysplit up into very minute thin fibrils, resembling cilia. The outer wall of each gland cell is extremely thin. A prominent but moderately staining nucleus is present on one side. The threads inside stain uniformly dark and hence the details of their structure are difficult to be made out. The function of these glands seems to be adhesive and, as has been mentioned, the worms firmly attach themselves to the substratum by means of the hind end. It is possible that the thin thread-like bodies inside the glands are some thick, sticky secretions which might enable the worm to firmly adhere to sand grains or other substratum. A pair of anal cirri is also attached to the posterior extremity of the anal segment. They are not much swollen at the base and imperceptibly taper to the tip.

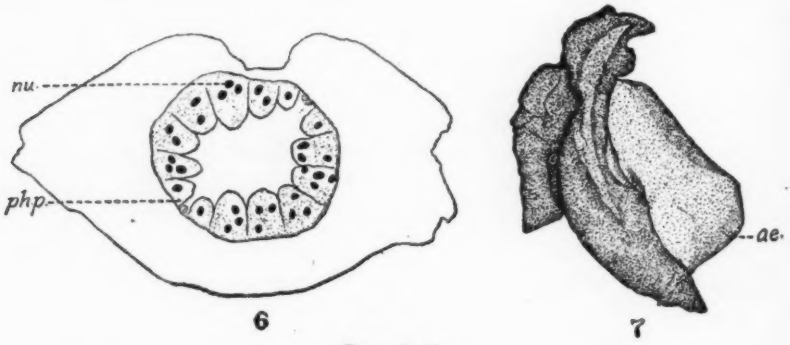
Body Wall

Transverse sections passing through the anterior, middle and posterior regions of the body are almost circular in outline and hence the body is cylindrical in shape. Forming the external layer of the body wall is the epidermis which is covered over by a thin cuticle. The epidermis consists of a single layer of flattened cells the nuclei of which stain poorly. Cutaneous glands are richly developed in the thickness of the body wall but are mostly confined to the sides of the body (Fig. 20). They are of various shapes being tubular, curved and coiled in different ways. They are of a mucus secreting function and the secretions probably enable the worm to adhere to sand grains and pebbles when the substratum is constantly being disturbed by the waves. The adhesive nature of these glands is shown by the readiness with which the worms attach themselves to the sides of the pipette or to the bottom of the dish when disturbed. In the males there is a continuous and rich development of these glands in the mid-ventral line of the body. Such a specialised row of glands is absent in the females. The epidermis is followed by an extremely thin layer of circular muscles which can be made out only under high magnification. Below this is the well developed longitudinal muscles, arranged in four bands, two dorso-lateral and two ventro-lateral. The dorso-lateral bands are situated close together so that

often they touch each other. The ventro-lateral muscle bands are thicker and larger than the other two and near the ventral nerve cord they show a tendency to turn inwards. In the other two species of *Praegeria* the corresponding muscle bands are better developed and the tendency to turn inwards at the border is more pronounced. A further advance in the development of these muscles has been observed in the case of *Pisionella indica* (Aiyar and Alikunhi, 1940)—an allied genus—wherein this turning inwards is complete, the inwardly turned edge coming in contact with the opposite end of the muscle band, thereby enclosing a space. Towards the anterior edge of the body, in the region of the pharynx, the longitudinal muscles are poorly developed and inconspicuous. An epithelial lining, the coelomic membrane, forms the innermost layer of the body wall.

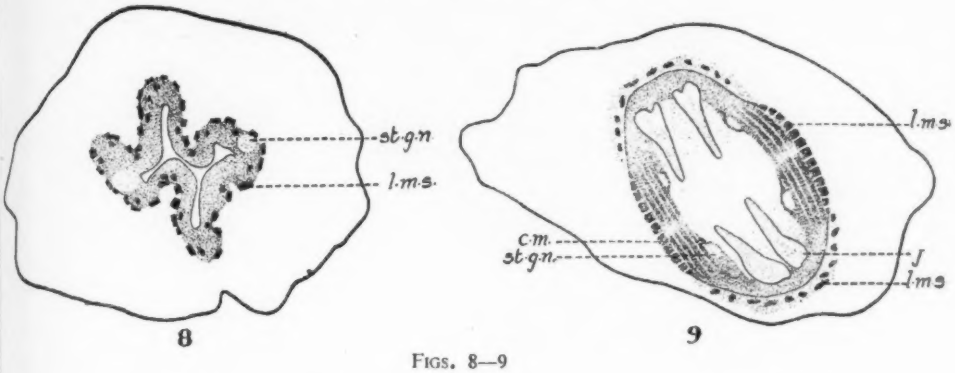
Alimentary Canal

The mouth is median and ventral and is supported by a muscular lower lip. It leads into an œsophagus which extends into the third setigerous segment (Fig. 1). This is followed by a highly muscular protrusible pharynx which normally extends to the sixth segment. The pharynx is provided with a crown of 14 conical, pointed papillæ of almost equal size (Fig. 6). The pharyngeal armature consists of two pairs of chitinised jaws which are supported by broad foliaceous aeilerons (Fig. 7). Of these jaws



FIGS. 6—7

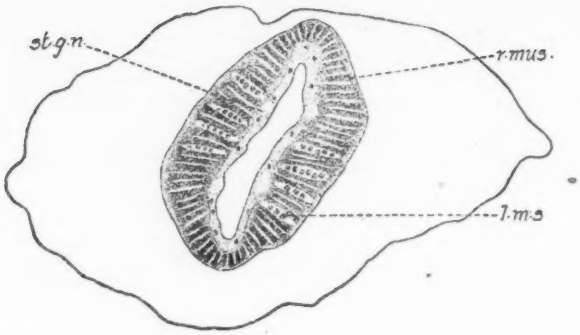
the outer one of each pair is provided with two or three blunt projections at the anterior pointed end (Fig. 7) while the inner one is devoid of such projections. The œsophageal wall is thin and around it are several bundles of longitudinal muscle fibres (Fig. 8), forming an almost complete layer. The inner epithelium in this region is constituted by fairly large protoplasmic cells. At the level of the jaws a strong development of circular



FIGS. 8—9

muscles is found besides a discontinuous layer of longitudinal muscle bundles on the exterior (Fig. 9). The pharynx, extending from the level of the jaws to the 6th segment, is highly muscular. Internally there is a strong epithelial layer in which only a few nuclei can be made out. On the outside there is a fibrous membrane in which nuclei are not seen. Between these two layers run powerfully developed bundles of radially disposed muscles (Fig. 10). Attached to the hinder extremities of the jaws and running down slightly to the sides are a few longitudinal muscle strands which keep the jaws in position and probably regulate their movements. In transverse sections (Fig. 10) these muscles are seen to pass through the spaces in between the radial muscle bundle of the pharyngeal wall. From a little behind this region a layer of longitudinal muscle strands appears surrounding the pharyngeal wall. The inner epithelium here is more protoplasmic and the nuclei of the cells stain more deeply (Photomicrograph 3). The pharynx is followed by the stomach which extends to the 9th or the 10th segment.

It is thin walled and devoid of any constrictions and is surrounded by a layer of longitudinal muscles, arranged in bundles (Fig. 11). The digestive epithelium here consists of protoplasmic cells the nuclei of which take a comparatively deep stain. From the 10th segment onwards the intestinal portion gets intersegmentally constricted at the level of the septa. The inner epithelial wall of the intestine is formed of a single layer of large cells with deeply staining round basal nuclei. Externally there are a number of scattered, extremely minute longitudinal muscle strands. Circular muscles in the wall of the alimentary canal have not been detected even under high magnification. The anus is posterior and ventral and is situated just in front of the caudal glands. The posterior portion of the alimentary canal and the anus are richly ciliated.



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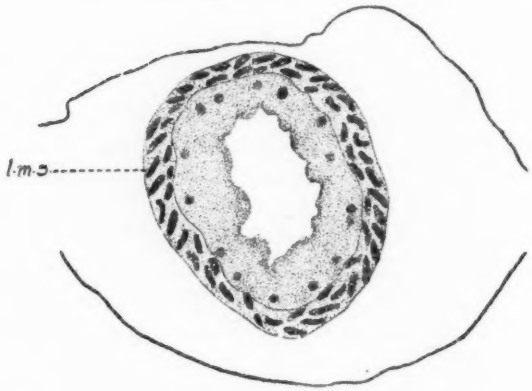


FIGS. 10—11

Nervous System and Sense Organs

Brain.—As has been mentioned, the brain is very anteriorly situated and is in the form of a pair of elongated tapering lobes, fused together in front and extending behind into the third setigerous segment. Anteriorly, the lobes fuse together after which they project as blunt processes and there is a groove in between (Figs. 12 *a* and 13). Posteriorly the lobes are far more separate and stand apart. The outer part of the brain is formed by a rind or sheath of ganglion cells while the inner region is made of the punctated substance which is best developed before the bifurcation into the posterior lobes. Transverse sections of this substance give the appearance of fine granules, interspaced with minute fibrils, while in longitudinal sections it has the appearance of extremely thin, wavy fibrils. Nuclei have not been observed in this substance, and it does not stain with iron hæmatoxylin.

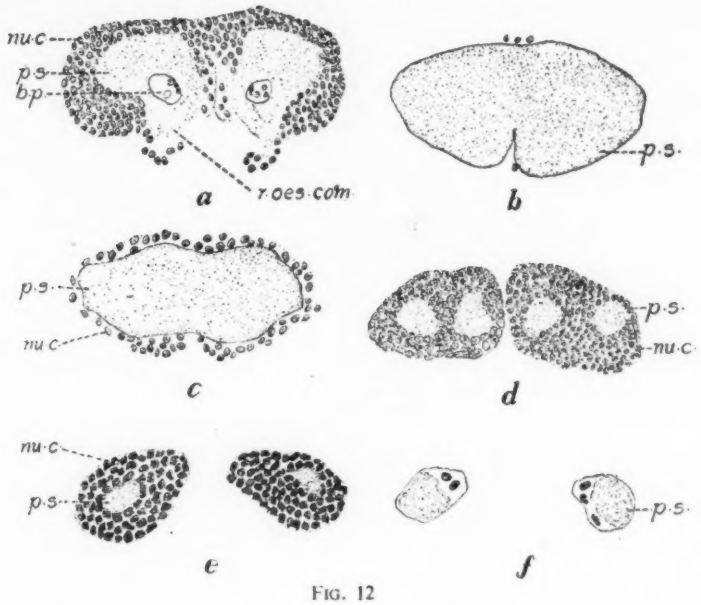


FIG. 12

The nuclei of the ganglion cells are very large and rounded and each occupies the major portion of the cell, the cytoplasm being much reduced. In sections stained in iron hæmatoxylin the nuclei stain moderately and are seen to be very closely aggregated. Towards the anterior end of the brain where the two lobes stand apart, these cells are few dorsally but are aggregated ventro-laterally. Proceeding backwards they are reduced on the ventral side and are grouped together dorsally near the median line, *i.e.*, in the anterior fissure between the two horns of the brain. At the point of union of the two anterior lobes the ganglion cells are present on the dorsal and lateral sides but are absent from the ventral side. Immediately behind the fusion of the anterior lobes the ganglion cells are completely absent from the outer aspect of the brain and dorsally the brain is in intimate contact with the hypoderm. In the middle portion of the brain only very few ganglion cells are present (Fig. 12 *b*). But further behind they again make their appearance (Fig. 12 *c*) and soon a thick covering of them is formed all round the brain. Here, in longitudinal as well as transverse sections, the central core, formed of the punctated substance, is seen to be divided into two parts due to the nucleated cells having encroached in the form of a bed in between (Fig. 12 *d*). The eyes are lodged in amongst the ganglion cells and just touch ventrally the upper surface of

the punctated substance in this region. The nuclei of the ganglion cells in the region behind the eyes are slightly larger and more closely packed. They take a deeper stain and are not rounded in outline (Fig. 12 *e*). The ganglionic sheath stops short some distance in front of the posterior end of the lobes where only the punctated substance is present (Fig. 12 *f*).

Many authors differentiate the polychæte brain into three regions which in the different families are believed to be homologous to each other. These regions correspond to the " Cerveau anterieur, cerveau moyen, and cerveau posterieur " of Pruvot and the " aire palpaire, aire syncipitale and aire nucale " of Racovitza and may be rendered as the anterior brain, middle brain and posterior brain, respectively. In *Praegeria* also the anterior horns, the middle fused region and the posterior lobes of the brain seem to correspond to the above three regions.

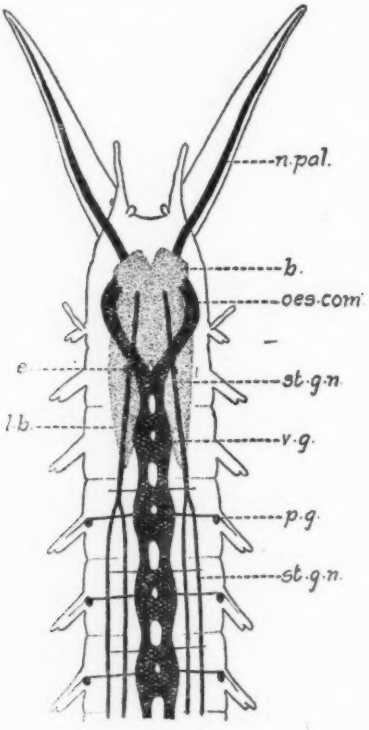


FIG. 13

The œsophageal commissures take their origin each by means of two roots from the inner core of the outer ventral aspect of the fused middle

region of the brain (Fig. 13). In forms like *Eunice torquata* and *Marphysa gravelyi* also the œsophageal commissures have been described as arising by means of two roots. In transverse sections it is seen that the inner core of the palps pass between the roots of the œsophageal commissures (Fig. 12 *a*). A few ganglion cells form a covering to the bases of the commissures but as they pass to the ventral side very few such cells are noticed. However, they are present on the posterior part of the commissures commencing from a little in front of the region where they fuse together to form the ventral nerve cord.

Ventral Nerve Cord.—The first pair of ganglia of the ventral chain is formed at the level of the eyes, *i.e.*, in the second setigerous segment where the two commissures fuse together. The posterior lobes of the brain project backwards well beyond the level of these ganglia. The ventral nerve cords fuse together at the ganglionic swellings but in the intervals they stand separate (Fig. 13). Well developed ganglionic swellings are present and whole mounts stained in Delafield's hæmatoxylin give a good picture of them. The ganglionic enlargement in each segment commences immediately behind the anterior septum and it is interesting to note that each pair of ganglia fuses together in two places, one immediately behind the septum and the other at the level of the parapodium. In front of the anterior septum the nerve cords are thin and devoid of ganglion cells (Fig. 14 *c*). A thick sheath

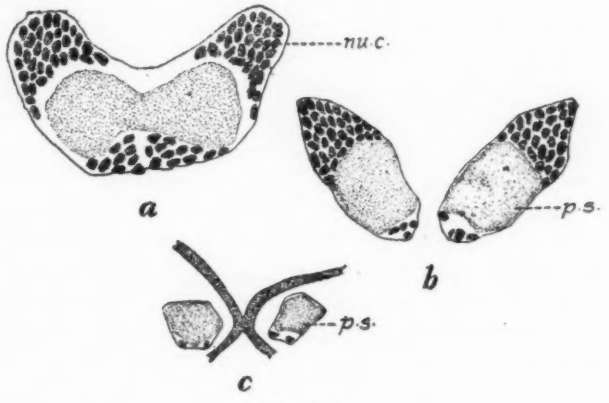


FIG. 14

of ganglion cells, usually arranged in three groups—two dorso-lateral and one ventro-median—is, however, present surrounding the punctated substance (Fig. 14 *a*). Where the two ganglia are separate, as well as immediately

behind the ganglia, the ventro-median group of ganglion cells is absent, and the dorso-lateral groups are in the form, of two conical lids (Fig. 14 *b*).

The fusion of the ganglia at two places reminds one of the condition of the ventral nerve cord in the Sabellaridæ, Serpulidæ, and Amphictenidæ, in which each segment is provided with two pairs of ventral ganglia, one in the anterior and the other in the posterior part of the segment (Hempelmann, in Kukenthal). It may also be mentioned that in *Pectinaria* Lamarck and *Petta* Malmgren even three pairs of ganglionic swellings are present in each segment.

Giant nerve fibres have not been noticed in the course of the nerve cord. A neural canal is absent. On the dorsal aspect of the nerve cords are inserted the bases of powerful oblique muscles, which in the hinder region of the segment pass between the nerve cords (Fig. 14 *c*). The ganglion cells are practically absent in this region.

Parapodial Ganglia and Nerves.—Well defined nerves are present supplying the parapodia and the palps (Fig. 13), the latter of which will be described in a subsequent section. The nerves to the first pair of parapodia arise from the œsophageal commissures while those to the second pair take their origin from the first pair of ventral ganglia. No other nerves could be made out in these two segments. In the succeeding segments, however, two pairs of nerves are seen to arise from the ganglia. The first pair takes its origin from the anterior part of the ganglia, immediately behind the septum, and are extremely slender nerves. In sections they can be traced only for a short distance between the muscles. The second pair arising from the posterior portion of the ganglia from the ventral aspect constitutes the parapodial nerves. They are much thicker than the anterior pair and, as usual, run between the longitudinal and circular muscle layers to the sides. At the outer border of the ventro-lateral longitudinal muscle band each of them enlarges into a ganglion—the parapodial ganglion. They are fairly conspicuous and in sections the ganglion cells are present in the form of a compact group towards the inner aspect, while the inner core or the punctated substance is situated close to the body wall. From each of these ganglia a slender nerve is given off to the parapodial lobe.

These ganglia correspond to the 'Ganglion de renforcement' of the French authors and from which nerves are supposed to be given off to the parapodia. The presence of these parapodial ganglia, though common in many families of Polychætes (Nereidæ, Amphinomidæ, etc.) is not universal.

Nerves to the sperm-sacs.—The ganglia of the segment bearing the sperm-sacs in the mature male worm are slightly larger than the others and as in the other segments two nerves are given off from the posterior ventral aspect of them. These nerves are distinctly thicker than the rest and the two lateral ganglia in which they enlarge are about the same size as the ventral ganglia (Photomicrograph 8). Unlike the condition in the non-genital segments, these nerves after the outer border of the ventro-lateral longitudinal muscle bands turn inwards to the body cavity with the result that the ganglia come to lie in a dorso-lateral position to the ventral nerve cord, attached to the ventral wall of the sperm-sacs. As in the parapodial ganglia the ganglion cells are grouped into a compact mass which, though much larger than in the former, forms a dorso-median cap to the punctated substance. From each of these ganglia a stout nerve is given off along the dorsal aspect of the sperm-sac, to the tip of the copulatory organ, but unlike the parapodial nerves ganglion cells are present along the course of these nerves. In transverse sections passing through the region of the sperm-sacs the origin of these nerves from the ventral ganglia, their course through the muscle layers and the ganglia at their tips can all be made out easily. A more or less similar condition of the nerve cord has been observed in the male genital segments of the other species of *Praegeria* also.

Stomatogastric Nervous System.—As in several polychætes a stomatogastric nervous system is present in this form also. A pair of slender nerves—the stomatogastric nerves—arises from the inner core of the ventral surface of the fused portion of the brain, almost at the same level as the roots of the œsophageal commissures (Fig. 13). These nerves proceeding ventrally become attached to the walls of the œsophagus on either side (Fig. 8). At the level of the jaws they are pushed more to the interior where each of them splits into two. The four slender nerves thus formed innervate the wall of the pharynx and come to lie external to the inner epithelium—two on either side of the lumen and each situated at some distance from the other (Figs. 9 and 10). In certain regions along the course of these nerves a few nucleated cells, similar to those on the outer surface of the brain, are present. Unlike the condition in *Marphysa gravelyi* (Aiyar, 1933) distinct ganglionic swellings are not visible but it seems that the presence of the nucleated cells along the course of these nerves probably indicates the position of ganglia, however inconspicuous they might be. Towards the posterior portion of the pharynx the two nerve strands on either side are seen to get united together by means of an elongated connective and beyond which they do not proceed.

Sense Organs.—The entire surface of the body is sensory in nature while the eyes, the palps, the elongated dorsal cirri of the buccal parapodia, the ventral cirri of the first pair of parapodia and the anal cirri also serve as sensory structures. As has been mentioned, the eyes are mere pigment spots in direct communication with the central core of the brain. In living specimens the palps are often moved about, probably feeling the surroundings and each of these is innervated by a stout nerve arising from the anterior

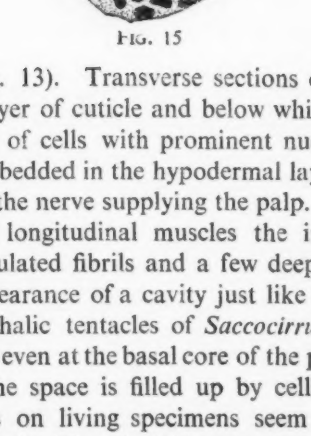


FIG. 15

horn of the brain (Fig. 13). Transverse sections of the palps show that there is an outermost layer of cuticle and below which is situated the hypoderm (Fig. 15) formed of cells with prominent nuclei and with very little cytoplasm around. Embedded in the hypodermal layer and in contact with the cuticle on one side is the nerve supplying the palp. Occupying the centre is a tubular sheath of longitudinal muscles the inside of which is filled up by a system of reticulated fibrils and a few deeply staining dark nuclei. This region has the appearance of a cavity just like the prolongation of the head cavity in the cephalic tentacles of *Saccocirrus* and *Protodrilus*; and this appearance is found even at the basal core of the palp. The nuclei present seem to indicate that the space is filled up by cells very probably loosely arranged. Osbervations on living specimens seem to show that there is a distinct lumen for the palp (Fig. 1) which extends to its very tip. From the base of the palp this lumen enters the head, enlarges slightly and then tapers to the posterior extremity which is situated between the base of the buccal spine and the first parapodium. This lumen is thickly packed by minute refractile corpuscles which are moved to and fro and which give the reticulate appearance in sections. The existence of such a closed cavity, though not so much specialised as in *Saccocirrus*, is highly interesting.

Nephridia and Genital Funnels

Nephridium.—The nephridia are very small and are present from the 6th setigerous segment onwards. As in *Pisionella indica* the nephridium

is a closed structure and the nephridial swelling projects conspicuously into the body cavity from the corner between the body wall and the septum

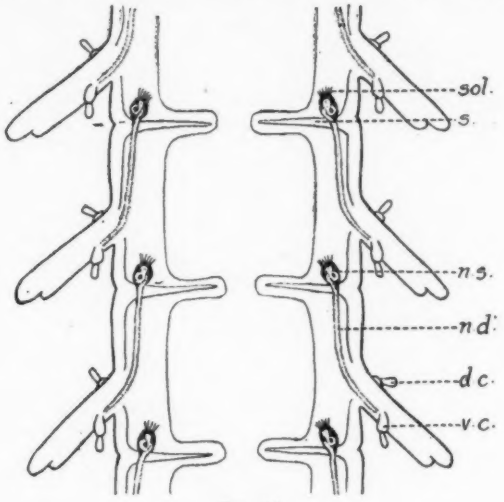


FIG. 16

(Fig. 16). The first two or three pairs of nephridia are distinctly larger and more prominent than the succeeding ones, and are provided with a larger number of solenocytes (about a dozen) which are arranged more or less fanwise, starting from the anterior part of the nephridial swelling. The nephridia are developed upto the last segment but in the posterior region they are much smaller and the number of solenocytes is much reduced. The nephridial swelling consists of a compact glandular cluster of cells which in the living specimens has a slightly yellowish tinge. A number of minute globules, probably of an excretory nature, are present in the wall of the nephridial swelling (Fig. 17). From the anterior portion of this swelling arise a few minute tubular structures representing the solenocytes. Each solenocyte has a slightly swollen base. The cell body is not conspicuous and there is no marked swelling at the tip. In sections the nephridial swelling is lightly stained while the solenocytes take deep stain. As in *Pisionella indica* the long flagella of the solenocytes converge and enter the central ciliated lumen of the nephridial swelling. The nephridial duct which immediately follows the lumen takes one or rarely two spiral loops (Fig. 17), pierces the septum and thereafter runs backwards straight and close to the body wall until it reaches the level of the parapodium when it bends outwards and finally opens to the exterior at the base of the ventral cirrus (Fig. 16).

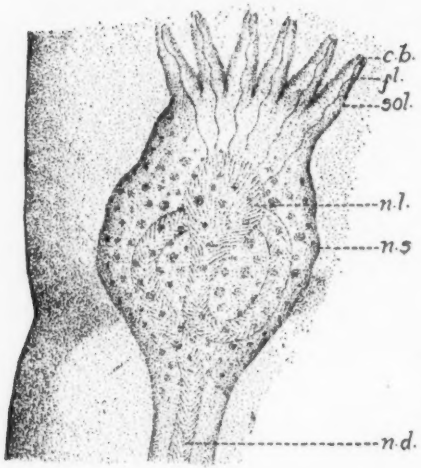


FIG. 17

Intra vitam staining with a weak solution of neutral red in sea-water was found to be very useful in following the course of the nephridium. In specimens stained for about half an hour the major portion of the nephridial swelling takes a deep red stain which marks out the cells clearly. The anterior extremity of the nephridial swelling as well as the solenocytes are poorly stained while the tip of the solenocyte takes deep stain and can be clearly made out as minute circular red spots. The wall of the nephridial duct being easily stained red, the central canal can be easily followed to its external aperture, situated at the base of the ventral cirrus. A comparison with the nephridial structure of *Pisionella indica* makes it evident that the nephridia of the present form differ from those of the former, in size, in the shape of the nephridial swelling and solenocytes, in the number and position of the solenocytes and in the coiled condition of the nephridial duct at its commencement.

Genital Funnels.—Ciliated organs or genital funnels begin to develop in the genital segments as the worm becomes mature and in fully ripe specimens they are in association with the nephridia. In a ripe female one or two pairs of genital funnels are developed, depending upon the number of ovarian groups. To take a specific example, in a worm with 34 setigerous segments two pairs of genital funnels were developed one in the 18th and the other in the 27th segment, each following an ovarian group and preceding a pair of receptacula seminis. In the males also the genital funnels are restricted to the reproductive region and usually only a single pair of them is developed in the testis bearing segment.

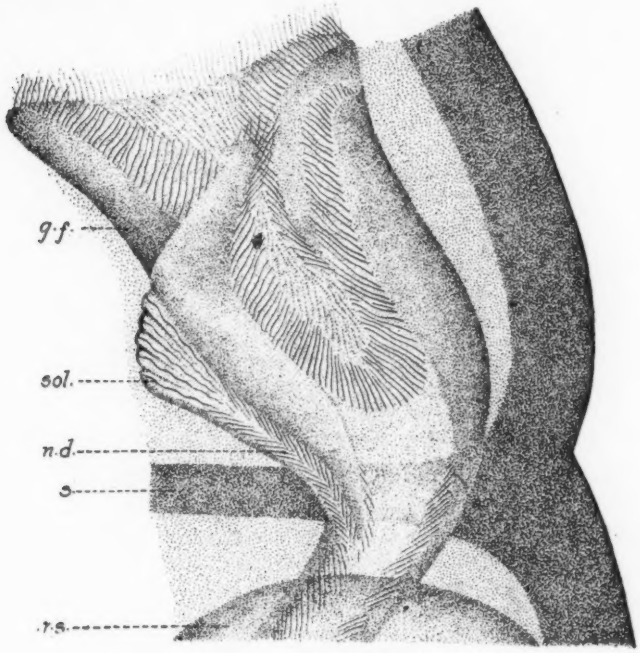


FIG. 18

The genital funnels are formed from the coelomic epithelium between the body wall and the septum of the particular segment in which they are found. When fully formed these funnels are quite distinct and are about 3 to 4 times the size of the nephridial swelling. In the females they are much larger than in the males. This larger size is probably necessitated by the bigger size of the ova that have to pass through them. The nephridial swellings with which these funnels are closely associated are highly reduced and can be made out only under high magnification (Fig. 18). The coiled condition of the lumen, mentioned as occurring in this region in the normal nephridium, has been lost and there is only a straight lumen. However, in transverse sections of 3 microns thickness the nephridial portion can be clearly made out from the genital funnel (Fig. 19). The nuclei in the wall of the nephridial swelling are few. They stain less deeply than the nuclei of the wall of the genital funnel and the nephridial portion as a whole is but lightly stained. The broad mouth of the funnel is dorsally directed, richly ciliated and clearly differentiated (Photomicrograph 4). The inner surface of the funnel is also richly ciliated. Its wall, though considerably thick,

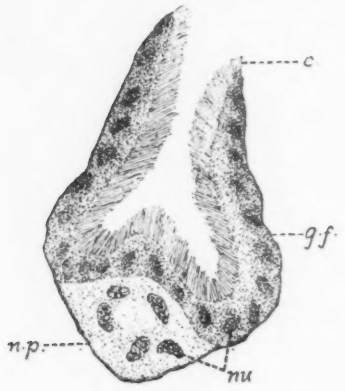


FIG. 19

is formed of a single layer of cells, with the nuclei more closely situated than in the nephridial wall. Transverse sections passing through the anterior one-third part of the ciliated organ is roughly in the form of a U, the dorsal lip projecting freely. The genital funnel is, therefore, in the form of a spatula with raised lateral edges which are fused behind. The funnel opens into the nephridial duct just at the point where the latter pierces the septum.

Reproductive System

Male: (1) *Testis*.—In the male the testis may usually be developed in any one of the segments between the 14th to 21st. It is in the form of a diffused mass of cells which in certain cases may extend to the next segment also (Photomicrograph 5). In transverse sections the testis is seen to encircle the alimentary canal which is pushed to the dorsal side (Photomicrograph 6). In the immature condition the testis is in the form of a pair of cell groups, one on either side of the median line, and attached to the anterior septum of the segment. As growth progresses the two groups enlarge and grow over the alimentary canal and encircle it (Photomicrograph 6) giving rise to the impression of a single structure in which distinction between the two contributing halves is lost. The whole mass is enclosed in a very delicate membrane. The testis cells are very large in comparison to the size of the worm and are also larger than those of *P. remota*. Towards the posterior extremity of the testis the cells are smaller with relatively smaller nuclei which do not differentiate so easily as those at the anterior part. The median or unpaired condition of the testis, in the adult, is seen in *P. remota* also, but in this latter case the anterior as well as the posterior extremities of the testis mass remain separate. The

mature sperms which are non-motile float freely in the body cavity or coelom of the segment bearing the testis and when the segmental chamber is filled with these liberated sperms they get pushed further into

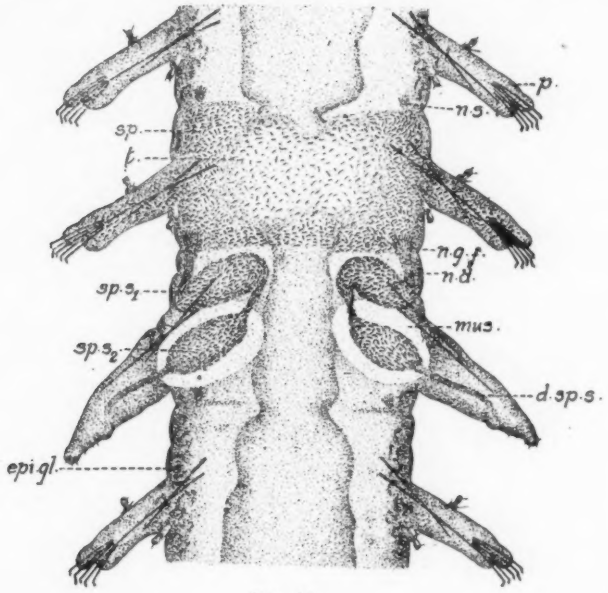


FIG. 20

the lumen of the parapodial lobe (Fig. 20). It is interesting to note that almost invariably the mature sperms are confined to the one segment containing the testis and only rarely are found in the preceding segment. In this feature the present form markedly differs from *Pisionella indica* in which the sperms freely pass into the neighbouring segments. The segment following the testis bearing one invariably carries a pair of sperm-sacs, the conspicuousness of which makes it one of the external distinguishing features of the male sex. The sperm-sacs are usually full of sperms and in the place of the normal parapodia of the segment there is a pair of complicated copulatory organs.

(2) *Sperm-sacs.*—The genital funnels in the testis bearing segment attract the mature sperms towards them by powerful ciliary action. In fact, in mature specimens the liberated sperms are very much crowded at the hinder part of the segment such that it is comparatively easy for them to get drawn into the genital funnels. The nephridial duct after piercing the septum runs down as a narrow tube and then takes a sharp curve upwards and forwards,

at the same time getting enlarged into a thin walled sac (Fig. 21). This sac again narrows into a short duct and bends downwards and backwards

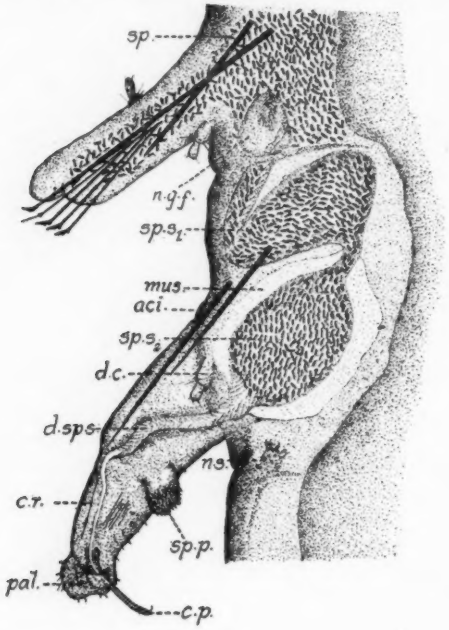
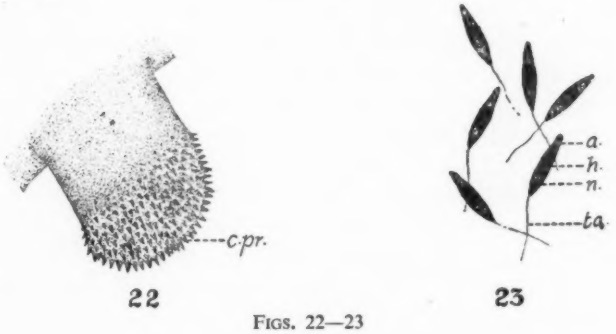


FIG. 21

and soon enlarges to form a second dilatation provided with a thick layer of muscles. The sperm-sac has a spacious lumen which is filled with sperms. The wall of the descending portion of the nephridial duct and that of the first saccular portion are much distended, thin, internally furnished with cilia and formed of a single layer of flattened cells. Transverse sections passing through the second saccular portion show a number of concentric rings of circular muscle fibres around the spacious lumen which is bounded by a single layer of ciliated cells (Photomicrograph 7). In contrast to *Pisionella indica* the structure of the sperm-sac is simple and the cells are devoid of any refringent granules. The sac again narrows into a duct which runs down and enters the copulatory organ (Fig. 21) to open finally to the exterior at the tip of a curved hook like papilla. The wall of this duct is partly cutinised and is ciliated at least in its proximal part. It will thus be seen that the sperm-sac actually consists of two separate dilatations, a feature in which it is widely different from the other two species of *Praegeria.*

(3) *Copulatory Organs.*—The copulatory organs are in close association with the sperm-sacs and it is noteworthy that invariably only a single pair of them is developed in each individual. In this feature the form more or less resembles *P. remota* but markedly differs from the third species, *P. complexa*, to be described elsewhere. In the formation of these structures the two parapodia of the segment in which the sperm-sacs are situated are highly modified and partially suppressed, the two dorsal cirri alone remaining unaltered (Figs. 20, 21). The setæ have disappeared and the two acicula alone persist. Each copulatory organ is broad at the base and gradually tapers to the tip where it is crenulate and covered over by stiff cilia. Behind the tip there is a prominent muscular, retractile papilla with the tip beset with a number of minute stiff cuticular projections (Fig. 22). At the tip of the copulatory organ which is supported by three or four cuticular rods, is a narrow, curved, hook-like, retractile structure, at the extreme end of which the efferent duct of the sperm-sac opens. This process is very small and when protruded stands erect, supported by the cuticular lining of its wall.



22 23

FIGS. 22—23

The condition of the copulatory organ in the other species of *Praegeria* is different. The completely retractile nature of the copulatory organ in *Pisionella* has been described and it may be noted that while in *Praegeria* the copulatory organs are always in the protruded condition, in the former they are protruded only at the time of copulation. Again, while in the present form the entire parapodium excepting the dorsal cirrus gets suppressed during the formation of the copulatory organ, in *P. remota* the copulatory organ is developed as a separate structure, the dorsal cirrus and an atrophied parapodial lobe persisting in the adult.

(4) *Sperms.*—In comparison with the size of the worm the sperms are very large and each measures 10 to 12 microns in length in the fresh

condition. Curiously enough, they are non-motile and do not exhibit any of the active movements so characteristic of sperms in general. Each sperm is elongated and slightly compressed from the sides so that when viewed from above it has a pointed spindle like appearance, while when on the side it presents a blunt anterior extremity. The tail or flagellum is extremely slender being visible only under very high magnification, and is usually of the same length as the preceding portions of the sperm or even slightly longer. Each sperm is divided into four parts (Fig. 23), the acrosome, the head, the neck and the tail. The acrosome is of almost the same length as the head, measuring about 2·3 to 2·5 microns and has a broad blunt anterior extremity. In sections and in smear preparations it takes only light stain. The head or nuclear portion in fresh preparations is more refractile than the other regions, is almost oval in outline and takes a deeper stain than the acrosome. It is, however, interesting to note that the neck region which follows the head is highly conspicuous and is as long as, or even slightly larger than the head and this region takes a light stain as the acrosome. In sections of materials fixed in Flemming's fluid without acetic and stained in iron hæmatoxylin the acrosome is in the form of a bluntly conical cap-like lid lodged on the anterior margin of the head and is moderately stained (Photomicrograph 8). The nucleus is in the form of a deeply stained circular area slightly compressed from the sides. Immediately behind the head at the commencement of the neck region is a minute circular spot—the centriole. The axial rod of the flagellum has not been found to extend to the centriole through the neck. On treatment with an aqueous (sea water) solution of eosin the sperms are seen to swell up and attain a rounded shape. The nuclear portion is surrounded by a vesicle like structure and its posterior half is more deeply stained than the anterior. In sections and smears the nucleus takes a uniform stain.

Early Stages of the Male Reproductive Organs.—The development of the complicated copulatory organs, sperm-sacs and genital funnels has been traced by examining a number of worms of varying stages of sexual maturity. As a rule in the segment destined to contain the sperm-sacs in the adult, the parapodia are not fully developed even at the beginning. In place of the parapodium a more or less foliaceous structure (Fig. 24) slightly bifid at the tip, is formed. This is supported by two acicula which persist in the adult. The dorsal cirrus of the parapodium is developed as usual but the ventral cirrus is absent. Of the two processes at the tip of the foliaceous structure the upper one is bluntly conical and probably represents the main lobe of the parapodium. The acicula are placed in this structure. The other process is more pointed and ends in a papilla at the tip of which the nephridial duct

opens (Fig. 24). A little distance behind the tip there is a conspicuous muscular knob representing the spinous papilla in the adult. At this stage sperms are not seen in the body cavity but still the testis is found to be

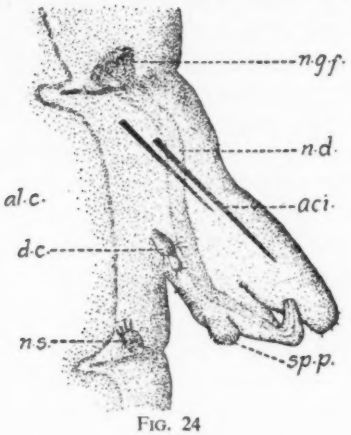


FIG. 24

developed. The genital funnel has not reached the adult condition. It has, however, established communication with the nephridial canal which is considerably wider than in the other segments and has now lost its previous coiled condition. After piercing the septum the nephridial canal runs down straight into the developing copulatory organ without any bend or curve in its course. However, the development of circular muscles half way down the nephridial duct, *i.e.*, at the portion which in the adult will from the second saccular dilatation of the sperm-sac, can be detected on closer observation. At this stage, therefore, the nephridial duct as a whole, has not undergone much change, save a general widening.

In specimens more advanced in development the genital funnels have assumed larger dimensions and have developed longer and larger number of cilia. The middle portion of the nephridial duct, *i.e.*, the second dilatation in the adult, with its surrounding muscles, has become conspicuous. The part of the duct leading to the exterior in the copulatory organ is bent and has assumed the adult characters. The copulatory organ has further elongated and is more pointed. In the posterior part of the testis mass fully formed sperms could now be seen but they are not liberated. In the next stage the nephridial duct just behind the septum enlarges into the first dilated part of the adult sperm-sac. The nephridial duct has thus been modified into the sperm-sac and has now more or less reached the adult

condition, but has not been filled with sperms. At a later stage sperms get liberated into the body cavity and are drawn into the sperm-sacs by the ciliary action of the genital funnels.

It may be noted that the copulatory organ, even though in its early stage of development has a bifid appearance, gets itself transformed into a conically pointed, entire process in the adult. From the structure of the adult copulatory organ it is probable that the blunt papilla in the developing copulatory organ gets gradually suppressed as development proceeds and that only the pointed papilla carrying the nephridial duct develops further and gets elaborated into the adult structure. It might, therefore, be that the suppressed papilla represents the parapodial lobe in the early stages and which is, however, not indicated in the adult except by the presence of the two acicula.

Female.—In the females also the gonads are developed in the posterior half of the body. Usually the ovaries are developed in definite groups after the 13th or the 14th segment. They are in the form of diffused clusters or groups of cells which are usually paired. In certain worms they may be developed only on one side. When developed on both sides in sections

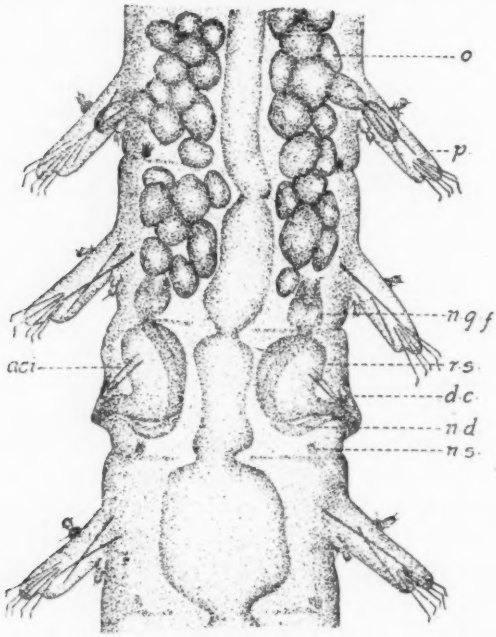


FIG. 25

they are seen to surround the alimentary canal (Photomicrograph 9) and are covered over by a delicate membrane. Each ovarian group extends into three to ten or fifteen consecutive segments and often one or two such groups may be developed. Immediately following each ovarian group there is a pair of receptacula seminis (Photomicrograph 10) which are spacious sacs usually filled with sperms. A pair of genital funnels is developed in the last segment containing ova (Fig. 25). To take a specific example, in a specimen with 34 setigerous segments two groups of ovaries were found developed. The first group occupied the segments 14 to 18 and a pair of receptacula seminis was present in the 19th segment. The second group of ovaries occupying the segments 23 to 27 was also immediately followed by a pair of receptacula seminis in the 28th segment. There were two pairs of genital funnels, the first pair in the 18th and the second, in the 27th segment. In quite a large number of worms only a single ovarian group is developed and as such only a single pair of genital funnels and receptacula seminis.

The receptacula seminis are thin walled sacs there being only a single layer of flattened cells forming their walls (Photomicrograph 11). They are blind internally and each sac is probably developed as described by Goodrich (1930) for *Pionosyllis neapolitana*, as an invagination of the body wall In the earlier stages the cavity of the receptaculum seminis is clear and spacious (Fig. 26). The nephridial duct can be clearly seen to open into the posterior portion of the developing receptaculum seminis, where there is a narrowing in the lumen. Its wall is considerably thick at this stage. The internal cavity is completely devoid of cilia and this fact points to a possible separate mode of origin of this cavity, unlike the sperm-sacs. In the later stages of development the portion of the receptaculum seminis where the nephridial duct opens, gets elongated into a duct with the result the nephridial duct and the receptaculum seminis open to the exterior by means of a common aperture.

When filled with sperms the receptacula seminis occupy the major portion of the segment in which they are placed. They are contractile in nature and their external ducts are provided with muscular walls. The parapodia of the segment carrying the receptacula seminis are completely atrophied and the setæ are absent (Figs. 25 and 26). In the place of the parapodium there is only a blunt stump supported only by a pair of minute acicula. The common external opening of the receptaculum seminis and the nephridial duct is situated at the tip of this stump. In the absence of parapodia in the segment bearing the receptacula seminis the present form markedly differs from the other species of *Praegeria* wherein the parapodia

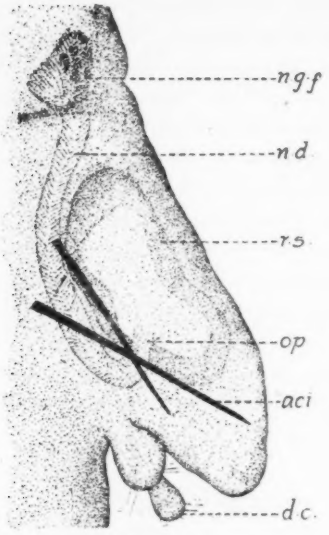


FIG. 26

of the like segments are unmodified. The nephridial duct in the genital segment is much dilated and after piercing the septum runs down close to the receptaculum seminis, bends outwards and joins the distal part of the duct of the receptaculum seminis. Several ova become mature simultaneously and are found to crowd at the posterior end of the ovarian group where the genital funnels are situated.

As has been mentioned in the beginning the ova are greenish in colour, large and with big transparent nuclei. By means of the dilated nephridial duct the mature ova, gathered in by the active vibrations of the cilia of the genital funnel, are taken to the exterior. The actual process of fertilisation has not been observed but from the union between the nephridial duct and the duct of the receptaculum seminis terminally and the presence of a single external aperture it can be inferred that the passage of the ova down the nephridial duct acts as a stimulus for the contraction of the receptaculum seminis and the consequent discharge of the sperms stored in there, with the result the ova get fertilised at the point of extrusion. The fertilised eggs soon commence development in the surrounding medium.

Sperms from the receptaculum seminis (*Spermatheca*).—The structure of the sperms found in the receptacula seminis of the female is very peculiar (Fig. 27) and different from that of the sperms present in the spermsacs of the male. It is quite obvious that the sperms are received into the

receptacula seminis at the time of copulation and the structure of the sperms at the time of transference should normally be the same as that described in the male. The differences observed should, therefore, have been acquired while inside the receptaculum seminis. The sperms do not form spermatophores. When pressed out they are all perfectly separate and as in the male they are also non-motile. But the head of the

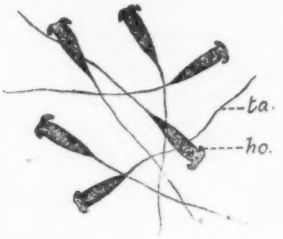


FIG. 27

sperm has undergone important transformations. Each sperm has developed a more or less oval shaped—sometimes circular—hood at the anterior extremity (Photomicrograph 12), thereby acquiring a nail shaped appearance. The hood measures about 3·3 microns along the longest part and is about 0·83 microns in thickness. At the outer margin the hood turns inwards a little. In the middle or the centre of the hood externally, there is a slight depression. When viewed from the sides this terminal hood gives the sperm a more or less anchor-shaped appearance and also the central depression in the hood is seen more clearly (Fig. 27). Immediately below the hood the body of the sperm is slightly narrow in the form of a short neck, but after this the body gets swollen in the form of a thick spindle which tapers suddenly. From the hood to the posterior extremity of the body the sperm measures about 6 microns. The swollen body is followed by an elongated slender tail measuring about 16 to 20 microns, *i.e.*, about three times the length of the body. In sections of Flemming fixed material stained in iron hæmatoxylin the structure of these sperms can be easily made out (Photomicrograph 12). The anterior hood takes only a very light stain, so that it is just indicated while the nuclear portion is darkly stained. From the nature of the staining property of the hood and that of the acrosome of the sperm in the male, it is suggested that the hood is a modified representation of the acrosome.

From the measurements of the sperms from the receptaculum seminis of the female it is seen that the head region is of almost the same length as that of the sperms from the male, while the tail, besides becoming more

prominent and thick, is considerably more elongated and is even longer than an entire sperm from the male. The more complicated structure and the increase in size of these sperms are important features which show that the sperms have not been undergoing any deteriorating changes inside the receptaculum seminis, but were undergoing some modifications, which have to be assumed as having some definite purpose in the act of fertilisation that is to ensue.

A large number of female specimens in various stages of sexual maturity were examined in the hope that some explanation to this curious phenomenon might be found. It has thus been observed that copulation takes place between a mature male and a rather immature female, judged by the minute size of the ova and the full development of the receptacula seminis in the copulating female specimens. In some of the specimens that were not fully ripe the receptacula seminis contained sperms identical in structure with those found in the males. In view of the changes, already described, undergone by the sperms inside the receptacula seminis, it might be inferred that in these specimens copulation had taken place only recently and sufficient time had not elapsed for any change to take place in the structure of the sperms. In certain other specimens that were also not fully ripe, the receptacula seminis contained both typical (as described in the male) as well as hooded sperms. It is, therefore, believed that the sperms at the time of copulation (in the male) are not ripe enough to effect fertilisation and have to undergo the above mentioned changes, while inside the female, to reach complete maturity. Though the condition is greatly different, it may be observed in this connection that the sperms in most or perhaps in all the Oligochæta do not mature in the testis or even in the body cavity, but they are received into the sperm-sacs where they ripen, before being used during copulation.

As to when these changes commence after copulation and the time taken to reach the final nail shaped stage, are points I have not been able to settle, since the worms have not been observed to copulate in the Laboratory. However, when mature female specimens are kept in the Laboratory they shed all the ova developed in a few days' time. This is soon followed by the complete disintegration of the genital funnels and the receptacula seminis and the development of fresh parapodia on the segments that contained them. All these organs have been found to be developed afresh when the ova develop again. Since sperms of the kind found in the males have been noticed only in the immature females and such sperms have never been found in the mature females containing ripe eggs, it seems highly probable that sperms are received by the females just prior to maturity and such sperms

modify themselves quickly and last only for a single period of ovarian activity, after which when regeneration of the sexual organs takes place, a fresh supply of sperms has to be taken. In this connection the condition in the Insects may be recalled, wherein in many cases copulation takes place only once in the life-time of an individual (Imms, 1934) and the sperms transferred into the spermatheca retain their motility and vitality for a considerable period. In the honey-bee itself the sperms in the spermatheca have been found to be functioning an year after copulation.

The role of the terminal hood remains a matter for conjecture. But I have often observed some of the sperms, when pressed out, attaching themselves to the partially everted wall of the duct of the receptaculum seminis by means of their hooded ends. The depression in the centre of the hood might facilitate this adhesion. As has been mentioned, motility of the sperms is absent unlike other polychætes with receptacula seminis. It might therefore be that the hood is a sort of an adhesive structure which coming in contact with the surface of the ovum readily attaches itself to it and thus ensures fertilisation, this being a possible means by which a strict economy in the use of sperms is maintained in view of their limited number inside the receptacula seminis and the large number of ova to be fertilised by them.

The non-motile condition of the sperms has been found in certain other groups of animals also, like the Decapod Crustacea, certain Myriapods (Chilognatha) and Arachnids (Palpigrada). Amongst the Decapods the spinous projections of the sperm are adhesive in nature and according to Koltzoff (as quoted by Wilson, 1925) fertilisation is affected by the action of an explosive capsule which is carried by the spermatozoon. As regards changes undergone by the sperms, formation of spermatophores is not uncommon in the Molluscs, Insects and Annelids. In many instances receptacula seminis are developed for the storage of spermatozoa which have been converted into spermatophores at or before the time of copulation. In *Pionosyllis neapolitana* Goodrich mentions the presence of spermatophores inside the receptacula seminis, in the female segments. In *Saccocirrus* and *Microphthalmus* paired receptacula seminis containing motile sperms inside are present in all the ova bearing segments, but the sperms have not undergone any change. Receptacula seminis are developed in the Alciopidæ and in certain Spionids (*Pygospio elegans*). In the Insects also sperms have been observed to be actively motile while inside the receptaculum seminis (Patton and Cragg, 1913).

Usually in the formation of spermatophores a number of sperms are enclosed in a common capsule, or attached together by their heads so that

individual sperms themselves do not undergo very considerable modifications. In the case of *Praegeria*, on the contrary, spermatophores are not formed but individual sperms undergo modifications while inside the receptaculum seminis, a feature in which it stands unique. Non-motility of sperms is extremely rare amongst polychætes but in *Pisionella* and *Praegeria* the sperms are found to be non-motile. In the light of this fact I am led to think that this will be the case, also in *Pisione*—the internal characters of which, unfortunately, are unknown at present and which is the only other genus of the family Pisionidæ. In this respect, therefore, the Pisionidæ should be considered unique. It is also probable that in the sperms of *Praegeria* when motility has been lost, the power of attachment by means of a hood, thereby ensuring fertilisation, seems to have been developed as in the Crustacea wherein the same result has been achieved in a totally different manner by the formation of adhesive spines and explosive capsules.

Along with the development of the genital elements certain internal changes also take place so as to accommodate the newly formed structures. In the ripe males as well as females in the genital segments the alimentary canal is in the form of a very narrow tube, pushed more to the dorsal side so that more coelomic space is available in the segments. The longitudinal muscle bands are very much reduced and especially in the sperm-sac bearing segment of the male they are extremely thin and inconspicuous. Though breeding takes place continuously, as evidenced by the availability of mature specimens throughout the year, it has been found, as already mentioned, by keeping the worms alive in the Laboratory, that the genital elements developed are all shed within a short period and after which the various organs developed in connection with reproduction are cast off. The details of these changes have been studied carefully in another species, *P. complexa* and an account of the same has already been given before the 28th Session of the Indian Science Congress, held at Benares in January 1941, and will form the subject matter for another paper.

Sexual Maturity and Position of Gonads.

The worms become sexually mature very early in their life history. Reproductive organs make their appearance when the worm has developed about 17 or 18 segments. Usually in the case of males, they become mature when there are about 20 setigerous segments. A comparison of several worms of different lengths shows that only a single pair of sperm-sacs is developed and that their position is always constant being situated in any one of the segments between 14 and 21. The worms continue to grow even after sexual maturity and fresh segments are developed at the posterior end. In a speci-

men with 20 segments the position of the sperm-sacs, situated in the 16th segment, is posterior. But in another specimen with 34 segments the position of the sperm-sacs, situated in the 16th or 18th segment, is in the middle region of the body. In another specimen with 48 segments the sperm-sacs were situated in the 20th segment. In this specimen, therefore, their position is a little in front of the middle region of the body. This can only be so as in polychætes new segments are added only at the posterior end. In the female the ova are developed only when the receptacula seminis are fully formed and the worms become mature when there are about 24 to 26 segments. As has been mentioned, the ovaries commence from segments varying from the 13th to the 18th. Unlike the condition in the males, here, the posterior segments are not sterile and a second group of ovaries may be developed after the first.

Summary and Conclusions

From the foregoing pages it is clear that the present form shows a number of interesting features in which it markedly differs from the other species of the genus. These characters seem to me of sufficient value for the creation of a new species for its reception and for which I propose the name *Praegeria gopalai.** The important characters of this species can now be summarised as follows:—

Praegeria gopalai n. sp.

Minute slender worm, 4 to 10 mm. long, with 25 to 50 setigerous segments; ventral cirrus of the buccal parapodium globular; ventral cirrus of the first setigerous foot slightly elongated; anal segment with two conspicuous groups of caudal glands; only a single pair of sperm-sacs, genital funnels and copulatory organs in the male; secondarily median testis enclosed in a membrane and often extending into two segments; tapering entire copulatory organ provided with a large spinous papilla sperm-sacs having double saccular expansions; in the female, one or two ovarian groups covered over by a membrane and each group extending into 6 to 15 consecutive segments; receptacula seminis and genital funnels corresponding to the number of ovarian groups; parapodia of the segment carrying the receptacula seminis completely atrophied and carrying only two acicula; sperms from the receptacula seminis nail shaped having developed a conspicuous terminal hood.

Locality:—Sandy Beach, Madras.

Holotype:—In the Indian Museum, Calcutta.

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* It gives me great pleasure in associating the specific name with Prof. R. Gopala Aiyar, Director, University Zoological Research Laboratory, Madras, at whose suggestion the study of the Fauna of the Sandy Beach, Madras, was undertaken.

Acknowledgements

I take this opportunity to express my deep sense of gratitude to Prof. R. Gopala Aiyar, for his valuable help and suggestive criticisms throughout the course of this study and to the University of Madras for awarding me a Research Studentship.

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EXPLANATION OF FIGURES AND PHOTOMICROGRAPHS

(All the figures have been reduced to ⅔ the original.)

FIG. 1. Anterior end of *Praegeria gopalai* n. sp. showing cephalic appendages. × 120.

,, 2. Parapodium of the tenth segment. × 600.

,, 3. Setæ from the tenth parapodium. × 1800.

(*a*) Simple seta. (*b*) compound seta with long terminal blade. (*c*) Compound seta with short terminal blade.

,, 4. Posterior end of the worm showing the structure of the anal segment. ×160.

,, 5. Transverse section through the caudal glands. × 900.

,, 6. Transverse section through the region of the pharyngeal papillæ. × 600.

,, 7. Jaw after treatment with caustic potash. × 600.

,, 8. Transverse section through the œsophagus to show the musculature. × 600.

,, 9. Transverse section of the pharynx at the level of the jaws. × 600.

,, 10. Transverse section of the pharynx passing through the region immediately behind the jaws. × 600.

,, 11. Transverse section of the stomach. × 600.

,, 12. Diagrams of sections of the brain showing the relationship between its lobes and the distribution of the ganglion cells. × 600.

(*a*) Section through anterior end of the brain showing the separate nature of the two halves.

FIG. 12. (*b*) Section of the anterior part of the brain showing the completely fused condition of the two halves and the absence of ganglion cells.

(*c*) Section of the hind region of the mid-brain. Note the reduction in the number of ganglion cells.

(*d*) Section through the middle of the posterior lobes of the brain.

(*e*) Section of the lobes of the brain behind the level of the eyes. Note the nature of the ganglion cells.

(*f*) Section of the lobes of the brain at the posterior extremity.

„ 13. Diagrammatic representation of the brain and the ventral nerve cord with their important branches. The podial nerves are shown only in three segments. The eyes have been indicated though on the dorsal side.

„ 14. Diagrams of sections of the ventral nerve cord.

(*a*) Transverse section showing the fused condition of the two halves at the ganglion. × 800.

(*b*) Transverse section after the first fusion of the ganglia. × 800.

(*c*) Transverse section after the ganglionic swelling. × 800.

„ 15. Transverse section of the palp. × 1350.

„ 16. Diagrammatic representation of the position and arrangement of the nephridia.

„ 17. Anterior portion of the nephridium from one of the middle segments. × 1800.

„ 18. Nephridial swelling and associated genital funnel of the female. Drawn from live specimen pressed under coverglass so as to show the reduced nephridial swelling. × 1800.

„ 19. Transverse section of the nephridial swelling and the associated genital funnel showing the intimate relation between the two. × 1350.

„ 20. Genital segments of a mature male showing the nature and distribution of the reproductive organs. Drawn from live specimen pressed under coverglass. × 200.

„ 21. Magnified drawing of the sperm-sac and copulatory organ of one side. × 400.

„ 22. Spinous retractile papilla of the copulatory organ. × 1350.

„ 23. Sperms from the male. × 1350.

„ 24. Developing copulatory organ. Note the bifid nature of the organ and the course of the nephridial duct. × 200.

„ 25. Genital segments of a mature female showing the distribution of the ovary, genital funnels and receptacula seminis. Drawn from live specimen pressed under coverglass. × 200.

„ 26. An early stage in the development of the receptaculum seminis. × 900.

„ 27. Sperms from the receptaculum seminis. × 1350.

PHOTOMICROGRAPH 1. Anterior end of *Prægeria gopalai* n. sp.

„ 2. Hind end of the worm showing the conspicuous caudal glands.

„ 3. Transverse section through the posterior region of the pharynx.

„ 4. Transverse section passing through a pair of genital funnels.

„ 5. Genital segments of a mature male. Note the presence of sperms in two segments immediately preceding the sperm-sacs.

„ 6. Transverse section through the testis.

„ 7. Transverse section through the middle of the sperm-sac bearing segment.

„ 8. Sperms from the male. Smear.

„ 9. Transverse section through the ovary showing the ova fully packed surrounding the alimentary canal.

PHOTOMICROGRAPH 10. Genital segments of a mature female showing the pair of receptacula seminis immediately behind the ovarian group. Note the condition of the parapodia of the segment bearing the receptacula seminis.

,, 11. Transverse section passing through the receptacula seminis.

,, 12. Sperms from the receptacula seminis.

KEY TO LETTERING IN TEXT-FIGURES AND PHOTOMICROGRAPHS

a.	Acrosome.	*n. d.*	Nephridial duct.
a.c.	Anal cirrus.	*n.g.f.*	Nephridial swelling and associated genital funnel.
aci.	Aciculum.		
ae.	Aeileron.	*n.l.*	Nephridial lumen or cavity.
al.c.	Alimentary canal.	*n.p.*	Nephridial portion.
b.	Brain.	*n.pal.*	Nerve to the Palp.
b.p.	Basal core of the Palp.	*n.s.*	Nephridial swelling.
b.s.	Buccal spine.	*nu.*	Nucleus.
c.	Cilia.	*nu.c.*	Nucleated or ganglion cells.
c.b.	Cell body?	*o.*	Ovum.
c.g.	Caudal glands.	*œs.com.*	Oesophageal commissure.
c.m.	Circular muscles.	*op.*	Opening of the nephridial duct into the receptaculum seminis.
cop.or.	Copulatory organ.		
c.p.	Cuticular papilla.	*p.*	Parapodium
c.pr.	Cuticular projection.	*pa.*	Palp.
c.r.	Cuticular rod.	*pal.*	Palpocils.
cu.	Cuticle.	*p.g.*	Parapodial ganglion.
d.c.	Dorsal cirrus.	*ph.p.*	Pharyngeal papilla.
d.c.b.p.	Dorsal cirrus of the buccal parapodium.	*p.l.*	Parapodial lobe.
		p.s.	Punctated substance.
d.sp.s.	Efferent duct of the sperm-sac.	*r.mus.*	Radially arranged muscles.
e.	Eye.	*r.oes.com.*	Roots of œsophageal commissure.
epi.	Epidermis.	*s.*	Septum.
epi.gl	Epidermal glands.	*sol.*	Solenocyte.
fl.	Flagellum.	*sp.*	Sperms.
g.f.	Genital funnel.	*sp.p.*	Spinous papilla.
g.sp.s.	Ganglion of the sperm-sac.	*sp.s1.*	First saccular portion of the Sperm-sac.
h.	Head.		
ho.	Hood.	*sp.s2*	Second saccular portion of the sperm-sac.
j.	Jaw.		
l.b.	Posterior lobe of the brain.	*st.g.n.*	Stomatogastric nerve.
l.m.	Longitudinal muscle.	*t.*	Testis.
l.m.s.	Longitudinal muscle strands.	*ta.*	Tail.
mus.	Muscular sheath	*v.c.*	Ventral cirrus.
n.	Neck.	*v.c.b.p.*	Ventral cirrus of the buccal parapodium.
n.c.	Nerve cord.		
		v.g.	Ventral ganglion.

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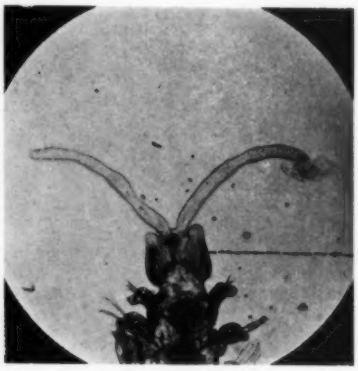

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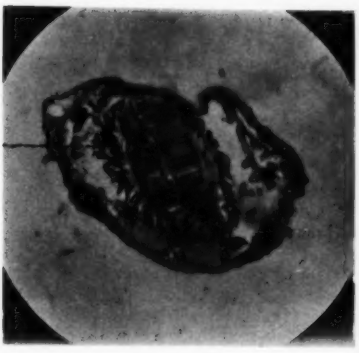

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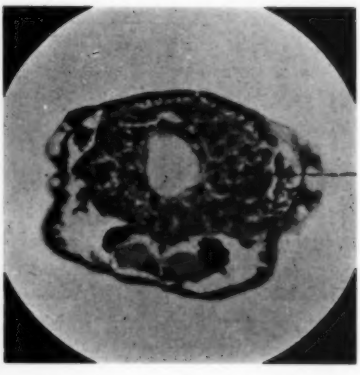

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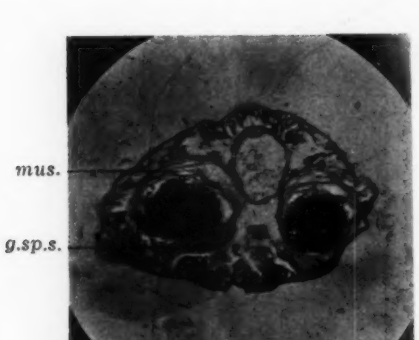

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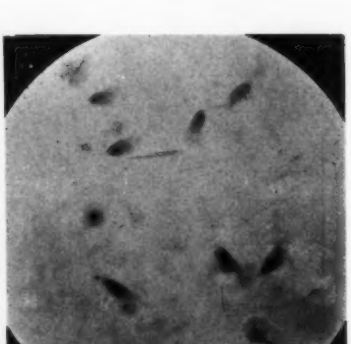

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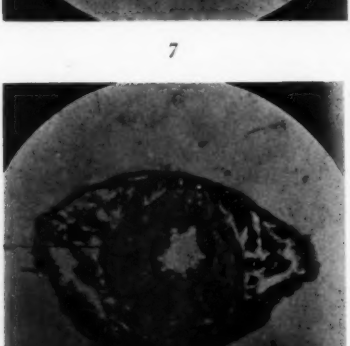

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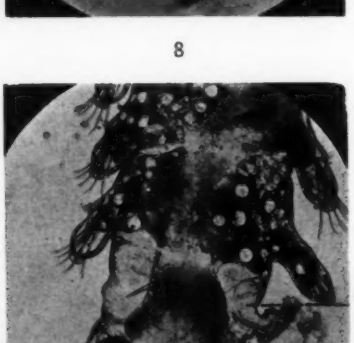

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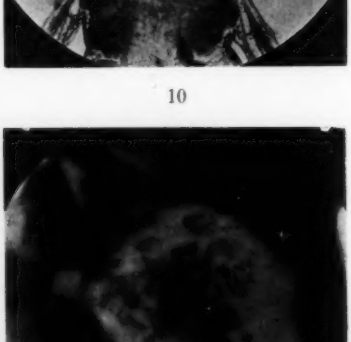

12

MORPHOLOGICAL AND CYTOLOGICAL STUDIES IN THE SCROPHULARIACEÆ

Part IV. The Development of the Embryo-sac and Endosperm in *Scoparia dulcis* Linn.

By Dr. T. S. Raghavan, M.A., Ph.D. (Lond.), F.L.S.
(*Head of the Department of Botany, Annamalai University*)
AND
V. K. Srinivasan, M.Sc.
(*Annamalai University*)

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I. Introduction

Scoparia dulcis is a tropical American plant now run wild and very common in India on waste lands and fallow fields. It is a glabrous undershrub about three feet in height. The flowers arise usually in twos from the axils of leaves. There are four fertile stamens.

II. Material and Methods

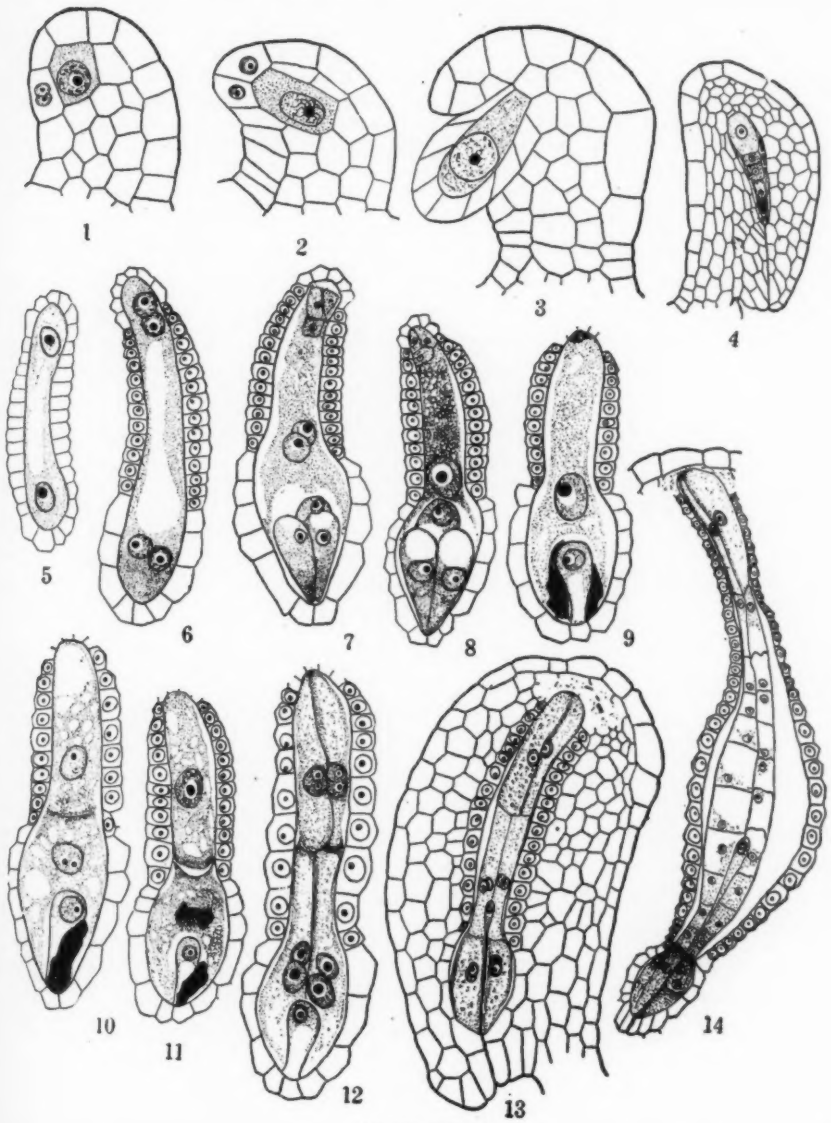
Material for the present work was obtained from plants grown in the University Botanical Gardens, Annamalainagar. Ovaries of various stages of development were fixed either in formalin acetic alcohol or in hot corrosive sublimate formalin acetic alcohol fixative. Sections were cut at thicknesses varying from 6 to 14 microns. All the preparations were stained in Haidenhain's iron-alum hæmatoxilin.

III. Observations

(*a*) *Megasporogenesis.*—The ovary is typically bicarpellary with indefinite number of ovules. The ovules are anatropous and are arranged on an axile placenta. The ovules arise as epidermal protrusions from the placenta. A hypodermal archesporium appears very early in the development of the ovule (Fig. 1). This hypodermal archesporial cell without cutting off any wall cell, increases in size and becomes the megaspore-mother cell. As the archesporial cell increases in size and elongates, the epidermal cells just above it undergo repeated anticlinal divisions to form a nucellar cap (Figs. 2 and 3). The single massive integument grows rapidly and at the same time, the ovule curves to assume the anatropous configuration. The megaspore-mother cell is surrounded by a single layer of nucellar cells (Fig. 3). The megaspore-mother cell enlarges considerably before its nucleus undergoes the heterotypic division to produce a dyad. The two cells by a second division form a linear tetrad of megaspores (Fig. 4). During the formation of the tetrad, the nucellar layer is pressed against the integument and it begins to degenerate. Its place is taken up by the innermost layer of cells of the integument, which becomes the tapetal tissue and appears as a row or band of cells on either side of the embryo-sac (Fig. 6). The chalazal megaspore of the linear tetrad is the functional one, while the three micropylar ones degenerate.

The nucleus of the functional megaspore divides into two to form a bi-nucleate embryo-sac (Fig. 5). Further divisions of these two nuclei result in the formation of the four and eight-nucleate embryo-sacs (Figs. 6 and 7). The mature embryo-sac is somewhat dilated towards the micropylar portion. The integumentary tapetum surrounds only the non-dilated chalazal portion of the embryo-sac (Fig. 8). The cells of the tapetum are of the usual shape and are uni-nucleate. Their function is to absorb nutrition from the cells of the massive integument and pass it on to the endosperm during the period of its formation and later to the developing embryo. The mature embryo-sac consists of two rather prominent synergids, an egg-cell, three antipodals and the two large polar nuclei (Fig. 7). Both the synergids and the antipodals are ephemeral and degenerate soon after fertilization.

(*b*) *Endosperm haustoria.*—In the post-fertilization embryo-sac the triple fusion nucleus is the most prominent and is situated near the oospore (Fig. 9). It undergoes a period of rest, after which it divides. This first division is transverse to the long axis of the embryo-sac. This transverse division is followed by a cross-wall, which separates the embryo-sac into



FIGS. 1–14

Fig. 1. Hypodermal archesporium. × 1500. Fig. 2. Same at a later stage. Note the anticlinal division of the epidermal cell. × 1500. Fig. 3. Megaspore-mother cell surrounded by a single layer of nucellar cells. × 1500. Fig. 4. Ovule showing the linear tetrad of megaspores and the degenerating nucellus. × 700. Figs. 5, 6, 7 and 8. Two-nucleate, four-nucleate,

eight-nucleate and seven-nucleate embryo-sacs. Note the tepetal layer. × 1200. Fig. 9. Post fertilization embryo-sac. × 1200. Fig. 10. Transverse wall separating a micropylar and a chalazal chamber. × 1200. Fig. 11. Shows the longitudinal division of the nucleus of the micropylar chamber. × 1200. Fig. 12. The two uni-nucleate chalazal haustoria and the micropylar chamber consisting of two tiers each of two cells. × 1200. Fig. 13. Ovule showing the differentiation of the four uni-nucleate micropylar haustoria. × 355. Fig. 14. Note the endosperm tissue, the elongated embryo, the tapetal layer and the chalazal and the micropylar haustoria. × 355.

two chambers, the micropylar and the chalazal (Fig. 10). The nucleus of the micropylar chamber now divides, this division being longitudinal (Fig. 11). A longitudinal wall is formed and the micropylar chamber is divided into two, by the longitudinal wall. The nucleus of the chalazal chamber also divides longitudinally, followed by wall formation. The resulting two cells, without any further division assume the rôle of the chalazal haustoria (Fig. 12). The nuclei of the two micropylar chambers now undergo a second longitudinal division, the plane of this division being at right angles to the first longitudinal division. As a result, the micropylar chamber consists of two layers of two cells each (Fig. 12). A transverse wall formed across these four cells results in the differentiation of a middle tier also consisting of two layers of two cells each (Fig. 13). The four cells towards the micropylar end, become the four micropylar haustorial cells, while the four cells of the middle tier, by repeated divisions build up the endosperm tissue. The two chalazal haustorial cells are long, narrow and tubular with their distal ends rather enlarged (Fig. 14). They elongate and absorb food materials from the chalazal region of the ovule. The resultant breaking-up of the cells in that region can be seen (Figs. 13 and 14). The four micropylar haustorial cells are comparatively less efficient as haustorial organs. The four haustorial cells later become a single four-nucleate haustorium, by the disintegration of the cross-walls (Fig. 14). In the present investigation, the method of development of the endosperm haustoria is thus found to be similar to that recorded previously in *Stemodia*, *Dopatrium* (Srinivasan, 1940) and *Ilysanthes* (Raghavan and Srinivasan, 1941).

(*c*) *Embryo*.—Most of the stages in the development of the embryo have been observed and they are quite normal and similar to the method of development described in *Ilysanthes parviflora* (Raghavan and Srinivasan, 1941).

IV. Discussion

There are practically no genera known in the Scrophulariaceæ where haustoria are not present. Schertz (1919) in his comprehensive study of *Scrophularia marylandica*, has mentioned that the genus *Scoparia* exhibited no endospermal haustoria. In an earlier paper of this series (Srinivasan,

1940) it was found that of a number of genera investigated, the genus *Angelonia* which had not received any attention till then, was found to exhibit no haustorial structures. Curiously enough, there was in this genus a highly interesting phenomenon shown namely the persistence of the synergids till a late stage of embryo formation. This phenomenon was however not seen in any of the other genera that were investigated and since this was found only in a genus where the usual haustorial formation was conspicuous by its absence, it was suggested that a correlation might exist between the absence of haustoria and the persistence of synergids. Other genera investigated since then also failed to reveal any such absence of haustoria such as was encountered in the genus *Angelonia.* In the literature on the Scrophulariaceæ, so far as we are aware, only the genus *Scoparia* is mentioned as exhibiting no haustorial structures (Schertz, 1919). But in that Schertz (1919) has made no mention of the synergids, their persistence or otherwise. Perhaps they escaped his observations. We were therefore, curious to find out whether the hustoria were absent and if so whether this phenomenon was in any way correlated to any abnormal behaviour of the synergids. It was with this intention that this genus was investigated. The chromosome number of this species has already been recorded by us in a previous paper (Raghavan and Srinivasan, 1940). The embryo-sac development has revealed that the formation of the endosperm haustoria is just like that in other genera investigated. Therefore, there can be no question of the absence of haustoria in the genus. The synergids are quite normal upto fertilization and degenerate soon after fertilization. There is no persistence on their part. It would thus appear that this case also proves at least in a negative manner the validity of the tentative suggestion made previously, of the existence of a correlation between the absence of haustorium and the persistence of synergids. So far only the genus *Angelonia* has exhibited this absence and revealed the correlation mentioned above. The only other genus namely *Scoparia* which was supposed to have no haustoria, has in the present investigation, been shown to be quite normal in the matter of haustorium formation. It would be interesting if future investigation on other genera would reveal any genus exhibiting no haustoria. In those few that may show this, the synergid behaviour should be watched with care to see how far the suggestion made previously could be generalised.

V. Summary

A hypodermal archesporium directly functioning as the megaspore-mother cell produces a linear tetrad of megaspores, the chalazal one of which produces a normal 8-nucleate embryo-sac. The synergids are normal and degenerate soon after fertilization.

The tapetum surrounds only the non-dilated chalazal portion of the embryo-sac.

There are two uni-nucleate chalazal haustoria and the four uni-nucleate micropylar haustorial cells later fuse into a single 4-nucleate haustorium.

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STUDIES IN THE CAPPARIDACEÆ

VII. The Floral Morphology of *Crataeva religiosa* Forst.

By Prof. T. S. Raghavan, M.A., Ph.D. (Lond.), F.L.S.
(Head of the Department of Botany, Annamalai University)
AND
K. R. Venkatasubban, M.Sc.
(Annamalai University)

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I. Introduction

This is a continuation of the series of papers on the morphology and cytology of some important Indian members of the Capparidaceæ (Raghavan, 1937, 1938 *a*, 1938 *b*, 1939 and 1941). The only paper that has appeared on this family since the publication of the above-named papers is that of Billings (1937). It deals with a very unusual type of embryo development of *Isomeris arborea*.

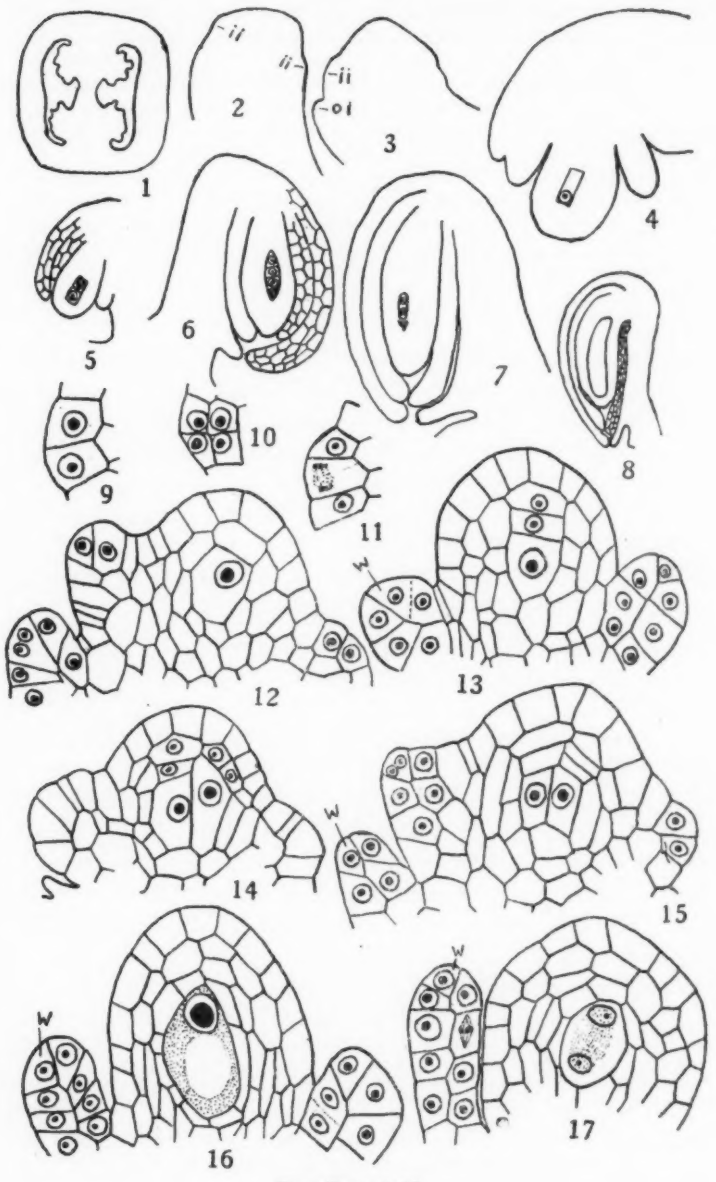
Cratæva is a Linnean genus named after Cratevas, an obscure writer on medicinal plants at the beginning of the first century B.C. There are ten species most of which are of medicinal value. The bark of *C. gynandra* (garlic pear) blisters like cantharides (Bailey, 1933). The bitter aromatic leaves and bark of *Cratæva religiosa* are used as a stomachic by natives (Oliver, 1868). The specific name presumably owes its origin to the plant being held sacred and as such are grown in temple compounds. *Cratæva religiosa* is commonly found in almost all the districts, although it is said to be a native of the Malabar coast.

It is a spreading unarmed deciduous tree of moderate size, flowering during the hot season when the plant is bare of foliage.

The present paper is a report of observations on the development of the microsporangium, and the macrogametophyte with incidental reference to the origin and development of the integuments.

II. The ovary

Typically the ovary is composed of two carpels by whose marginal fusion two parietal placentas result on which the ovules arise in about four rows (Fig. 1). In *Cratæva*, while normally the gynæcium is bicarpellary, there occur not infrequently ovaries which at first appear to be tricarpellary. Plate I, Figs. 1 and 2 which are photo-micrographs of young ovaries, show at A of Fig. 1, the incomplete marginal fusion of the adjacent carpels. In Fig. 2 a small crescent-shaped loculus has developed (A′), and this undoubtedly has resulted from the production of what may be termed a secondary tissue arising out of an incomplete fusion of the carpellary margins. For this reason, while the two lateral loculi bounded by the adjacent carpels, are equal in size, the loculus A′ is very much smaller, being merely the outgrowth tissue A of Fig. 1. The gynæcium therefore though tri-carpellary in appearance, is to be looked upon as only bi-carpellary, the placenta at B only being the normal production of the fusion of the adjacent carpellary margins, while B′ and B″ represent individual unfused carpellary margins. Further support is lent to this interpretation of the nature of these "abnormal" gynæcia by the occurrence of two separate vascular bundles at A of Fig. 1 indicating the unfused marginal strands of the carpels. Such gynæcia were also recorded in *Gynandropsis pentaphylla* (Raghavan, 1939) where, in addition, apparently tetracarpellary conditions were seen. In such cases diagonally placed carpels are equal in size and one set is much bigger than the other. The smaller loculi represent the secondary tissue arising out of a lack of fusion of the carpellary margins. While in tri-carpellary condition fusion has taken place on one side resulting in a normal placenta, in the ' tetracarpellary ' gynæcium no fusion has taken place on either side, so that the four placentas represent the unfused margins of the two adjacent carpels. A diagrammatic representation of this interpretation, has been given in the previous paper (Raghavan, 1939). These observations in another genus of the same family only go to confirm the classical bi-carpellary concept of the Capparidaceous gynæcium, based on a monomorphic interpretation. Further detailed ontogenetic and anatomical investigation, a report of which forms the subject-matter of a separate communication, also supports this concept.



TEXT-FIGS. 1–17

Fig. 1. T.S. of very young gynæcium at a lower level showing the replum-like septum. Note the ovules originating in four rows on either side of each placenta. × ca 75. Figs. 2 and 3. The

initiation of the integumental primordia; *ii.*,=inner integument; *oi.*, outer integument. × 750. Figs. 4–7. Development of the integuments, at megaspore-mother cell, dyad, tetrad and bi-nucleate embryo-sac stages. Fig. 4 × 750; the rest × 350. Fig. 8. Just after fertilization, the ovule begins the campylotropous curvature and also the inner integument becomes three-layered. × 150. Figs. 9–11. Integumental primordia and their behaviour; explanation in the text. × 1700. Fig. 12. Megaspore-mother cell having cut off the primary parietal cell. Note also the development of the integuments. × 1700. Fig. 13. Do two wall layers cut off periclinally. × 1700; *W.*, wedge-shaped apical cell of the integument. Fig. 14. Two juxtaposed megaspore-mother cells one of which has cut off two tangential wall cells, the other two anticlinal wall cells. × 1700. Fig. 15. Do. both the mother cells have cut off parietal cells by successive × 1700. Fig. 16. Megaspore-mother cell in synesis. × 1700. Fig. 17. Dyad formation. Periclinal divisions. Telophase of the heterotypic division. × 1700.

III. The origin and development of the integument

The primordium of the inner integument makes its appearance slightly earlier than the outer (Text-Fig. 2 *ii*) which soon arises as a protuberance on the convex side of the ovule (Text-Fig. 3 *oi*). Though the outer integument (*oi*) is slightly later in its origin, it soon overtakes the inner (Text-Figs. 4 and 5), and by the time the linear tetrad is organized it completely envelopes it (Text-Fig. 6). The halves of the inner integument come together some time later when the embryo-sac is in its bi-nucleate condition (Text-Fig. 7), and the massive micropyle thus formed is more or less straight and not zigzag as in *Cleome Chelidonii* (Raghavan, 1937).

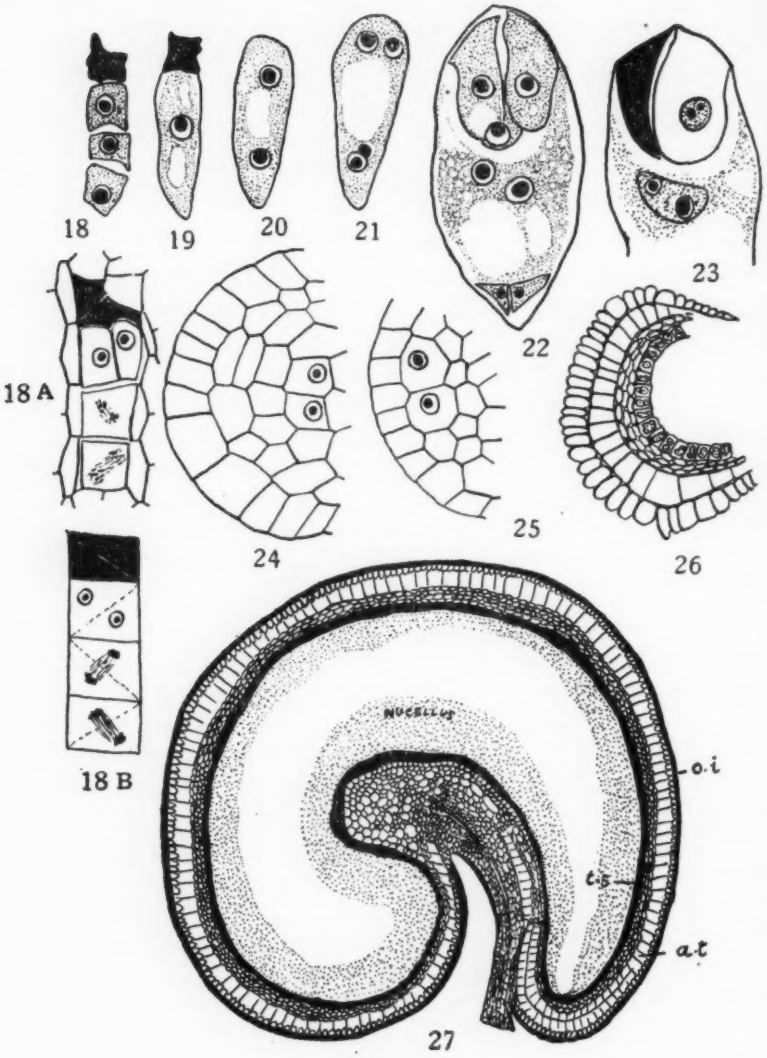
The integument initial is a superficial cell which by its larger size and richer protoplasmic contents can readily be recognised from the epidermal cells above which it protrudes slightly. This soon divides by a radial wall resulting in two juxtaposed cells bigger than the rest (Text-Fig. 9). By a tangential wall four cells are formed (Text-Fig. 10). One of the upper cells by an oblique wall cuts off a wedge-shaped cell. Sometimes the oblique wall-formation preceded the division of the juxtaposed cell (Text-Fig. 11). By a further series of tangential divisions a two-layered integument results (Text-Figs. 12–17), but the wedge-shaped apical cell always persists (*w*), till a comparatively late stage when essentially by its activity a massive micropylar region is built up. The outer integument remains two-layered throughout, while the inner integument till fertilization remains also two-layered and then becomes three-layered (Text-Fig. 8). In a mature seed (Text-Fig. 27) the two integuments forming the testa could yet be recognised. The outer integument (*oi*) is still two-layered of which the outer layer has become sclerotic. The inner integument is about six-layered of which the cells of the outermost layer are radially elongated and constitute the aqueous tissue (Text-Fig. 27, *a*, *t*) as in *Cleome Chelidonii*. The innermost layer lining initially the nucellus, and then the embryo is sclerotic and forms the tracheary sheath (*t*, *s*). These two layers of the inner integument doubtless play an

important part in the economy of the seed. The former is helpful in the storage of water in the initial stages of the development of the seed, while the latter, possessing as they do the structure of tracheids, function first as accessory water supply system for the embryo on account of the layers' close association with the vascular structure of the funicle, later acting as a sponge, absorbing moisture through the micropyle and constituting a kind of water jacket surrounding the embryo.

IV. Macrosporogenesis and macrogametophyte

The differentiation of the archesporium is slightly later than that of the integuments (Text-Fig. 12). The primary archesporium consists of a single hypodermal ro very often of two cells (Text-Figs. 14, 15). The primary parietal cell is cut off by the usual tangential division (Text-Fig. 12). Most usually this parietal cell cuts off by successive periclinal divisions a number of wall cells (Text-Figs. 13, 15), though occasionally anticlinal division follows the primary parietal cell division (Text-Fig. 14). About four layers of wall cells are organized before the megaspore-mother cell enters upon its division (Text-Fig. 16). Text-Fig. 17 shows the megaspore-mother cell in telophase of the heterotypic division. Linear tetrad is organized in the usual manner (Text-Fig. 18). T-shaped tetrads are also of frequent occurrence.

One very interesting case was found in which there were eight megaspores being formed instead of the usual four (Text-Fig. 18 A and Pl. XII, Fig. 6). And the interesting thing about them is that the planes of division of successive cells were at right angles to one another, so that the four walls of these eight cells formed a zigzag line. In other words, the two daughter cells resulting from the division of the mother cell were diagonally placed instead of vertically as is usually the case. This is diagrammatically represented in Text-Fig. 18 B. In Text-Fig. 18 A the uppermost two cells have degenerated. The next two cells have been fully organized while the remaining two are in telophase of division. No later stage than this was found and it is likely that these represent two superimposed linear or rather diagonal tetrads, having been organized by two megaspore-mother cells which were originally placed one above the other. Support is lent to this interpretation by the upper tetrads being in a more advanced stage of division than the lower. If this is accepted the embryo-sac formation must be considered as of the normal type belonging to the usual monosporic eight-nucleate kind (Maheshwari, 1937). If on the other hand all the eight cells are regarded as having originated from a single mother cell, then there will be one division more than in the normal type in reaching the eight-celled embryo-sac stage.



TEXT-FIGS. 18–27

Fig. 18. Linear tetrad with the micropylar megaspore degenerating. × 1700. Fig. 18*a*. The abnormal megaspore formation. Explanation in the text. × 1700. Fig. 18*b*. Diagrammatic representation of the same to show that the planes of division of successive cells are at right angles to one another. Note also that the top four cells are in a more advanced condition than the lower two. Fig. 19. Uni-nucleate embryo-sac. × 1700. Fig. 20. Bi-nucleate embryo-sac. × 1700. Fig. 21. Tetra-nucleate embryo-sac. × 1700. Fig. 22. Mature embryo-sac; same

as photographs, Fi s. 5 A and 5 B, Plate XII. × 1700. Fig. 23. Fertilization. The dark streak is the relic of the pollen tube. Of the two nucleoli in the egg cellulose belongs to the male cell. × 1700. Figs. 24 and 25. The archesporium of the anther consisting of two hypodermal cells which are seen to have cut off three layers of wall cells, while they themselves have not commenced to divide. × 1700. Fig. 26. Fully formed anther sac. Note the hypodermal endothecium composed of radially elongated cells, three layers of small thin-walled cells and the tapetum lining the anther sac. The nuclei of the tapetal cells are in various stages of division. × 350. Fig. 27. A mature ovule. Note the fusion of the funicle with the micropylar side of the ovule; *oi.*, outer integument; *a.t* = aqueous tissue; *t.s.*, tracheary sheath. ×75.

The further development of the embryo-sac conforms to the usual type laid down for dicotyledons (Text-Figs. 19–22). The chalazal megaspore functions (Text-Fig. 19) and the mature embryo-sac is of the normal type. The egg-apparatus consists of two prominent synergids in each of which the nucleus is located above a well-defined vacuole (Text-Fig. 22 and Pl. XII, Figs. 5 A and 5 B). They possess the "Hakenförmige Leistenbildung" (Dahlgren, 1928), a feature which was found in the other two Capparidaceous genera investigated, *Cleome* and *Gynandropsis* (Raghavan, 1937, 1938 *a*). It would appear to be a characteristic feature of the Capparidaceæ (Pl. XII, Fig. 7). The egg is normal with a prominent nucleus surmounted by a large vacuole (Text-Fig. 22). The polar nuclei are large and fuse comparatively late and the fusion nucleus takes up a position just below the egg. Text-Fig. 23 represents ostensibly a stage in fertilization, the dark patch representing the remains of the pollen tube, and one of the two nucleoli in the egg belongs to the male cell.

V. The microsporangium

The archesporium consists of groups of two hypodermal cells below each of the four corners of the anther (Text-Fig. 25). These cut off primary parietal cells (Text-Fig. 24) and these like those of the megasporangium are more active at first than the microsporagenous cells, so that about four layers of wall cells are organised while the sporogenous cells are yet in an undivided condition. In a mature anther about six layers of wall cells are organized (Text-Fig. 26) of which the innermost layer lining the anther sac functions as the tapetum. The epidermal cells become radially elongated, so do the cells of the hypodermal endothecium. The tapetal cells also become somewhat radially elongated while the intermediate three layers of cells being thin-walled, become crushed.

The tapetal cells are uni-nucleate initially, but very soon they become bi-nucleate even before the microspore-mother cells have entered upon their meiotic stages. Plate XII, Fig. 4 shows the tapetal cells in various stages of mitotic division. Soon they become tetra-nucleate but the nuclei seldom

remain separate (Pl. XII, Fig. 3). This confirms the careful results of Bonnet (1912). In the same tapetal layer one meets with almost all the conditions, bi-nucleate separate, tetra-nucleate separate and all stages of fusion of the four nuclei. After this the fusion nucleus gradually degenerates; so do the cells and presumably the contents are discharged into the anther sac to be utilized by the dividing microspore-mother cells. No definite periplasmodium was recognised. By the time homotypic divisions are completed the tapetal layer is completely disorganized.

VI. Chromosome number

The haploid chromosome number is thirteen. Plate XII, Fig. 8 shows a first metaphase plate. An account of the meiotic details has already appeared (Raghavan and Venkatasubban, 1939).

VII. Summary

The haploid chromosome number of *Cratæva religiosa* Forst., is thirteen. Further ontogenetic evidence based upon the occurrence of ' tricarpellary ' gynæcea and their interpretation, is adduced in support of the classical concept of the carpel and the bicarpellary nature of the Capparidaceous ovary.

The development of the macrogametophyte is described with a detailed description of the origin and development of the integuments right up to the mature stage of the seed.

The development of the microsporangium with details of the behaviour of the tapetal cells is described.

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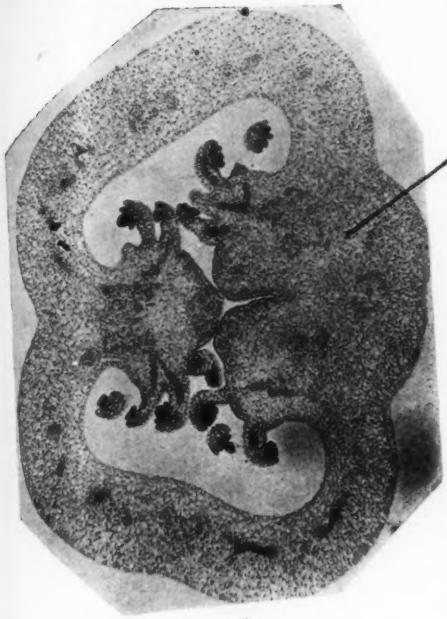

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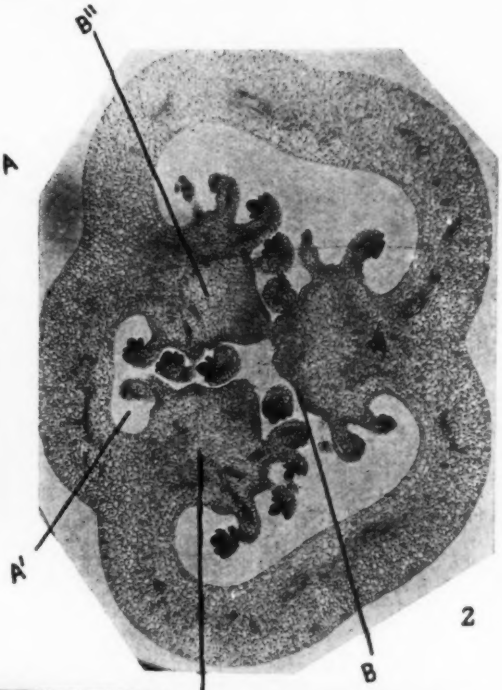

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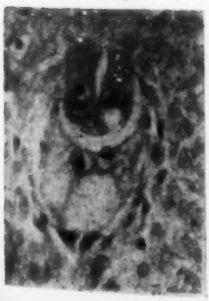
5 B



5 A

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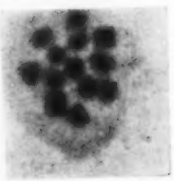

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EXPLANATION OF PLATE XII

FIG. 1. T. S. of young ovary basal portion. The tissue at A shows the incomplete fusion of the adjacent carpellary margins. Note also the two vascular strands of that region while on the opposite side they have fused to form a single bundle. × 85.

FIG. 2. T. S. of a slightly older ovary and at a slightly higher level. Loculus A′ is the outgrowth tissue A of figure 1. B is a normal placenta while B′ and B″ represent the unfused carpellary ends. × 85.

FIG. 3. Tapetal cells showing the nuclei in various stages of division and fusion, bi-nucleate, tetra-nucleate conditions being the most common. Note that the p.m.c. in its first metaphase. × 600.

FIG. 4. Tapetal cells in various stages of mitosis. × 600.

FIG. 5 A and 5 B. Fully developed embryo-sacs. The antipodals have degenerated; their remains could be seen only in 5 B; the polar nuclei have not yet fused. Note the prominent vacuole below the nucleus in the synergid. × 550.

FIG. 6. The abnormal case of megaspore formation. Explanation in the text. × 600.

FIG. 7. The synergids alone enlarged to show the Hakenförmige Leistenbildung. × 800.

FIG. 8. First metaphase plate greatly enlarged. Thirteen bivalents could be clearly counted × 5200.

THE INTERSTITIAL CELLS IN THE TESTIS OF *ICHTHYOPHIS GLUTINOSUS* LINN.

By B. R. Seshachar

(Department of Zoology, Central College, Bangalore)

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Nothing is known of the structure of, or the seasonal changes in, the interstitial tissue of Apoda and it is the purpose of this paper to describe in some detail the results of my study of this tissue in the testis of *Ichthyophis glutinosus*. For my work on the spermatogenesis of this animal I have been able to collect specimens throughout the year and am therefore able to present an idea of the changes occurring in the interstitial cells during the different seasons of the year.

Our knowledge of the interstitial tissue of the male Amphibia is itself meagre. Among the urodeles, the study of Humphrey (1921), and of Kolmer and Koppanyi (1923) are important. Champy (1913) and Aron (1921 *a*, 1921 *b*) have also described the changes occurring in the interstitial tissue and have discussed at some length the bearing of this tissue on the development of secondary sex characters. In some urodeles like *Molge cristata* (Aron, 1921) and *Pleurodeles waltli* (Kolmer and Koppanyi, 1923) this tissue becomes separated from the testis and lies beside it in the form of a distinct secondary body, which Aron found (in *Molge cristata*) closely related with the development of secondary sex characters. Among the Anura, the work of Friedmann (1898), Mazetti (1911) and Champy (1913) are important. Clearly, so far as the Amphibia are concerned, two definite schools of thought have come to being: (1) the first one including Friedmann (1898), Mazetti (1911), Aron (1921) and Kolmer and Koppanyi (1923) which believes that the development of interstitial tissue is parallel with the development of sex cells, and (2) secondly, the school which includes Champy (1913) and Humphrey (1921) arguing that the two have no relation with each other and that indeed, in some cases (*Rana esculenta*) the development of the two is never parallel.

This problem is closely related with the functional significance of interstitial tissue. Two functions have been assigned to it. First, the so-called 'trophic' function, which means that the interstitial cells prepare a material

which is necessary for the sex cells. The second and the more important function attributed to the interstitial cells is the function of the elaboration of hormones whereby they are believed to develop an internal secretion, which, among other things, marks the development of secondary sexual characters. The literature on this subject is extensive and so far as the Amphibia are concerned, the discussion between Champy and Aron has shown that there are really two sides to this problem.

From these points of view the case of *Ichthyophis* is very interesting. I have already reported (1936, 1937) that two definite phases in the activity of the testis can be recognised in this animal. From March till November, the testis is active and spermatogenesis is in full progress. During the winter months, the testis is at rest and the locules are empty. No stages of spermatogenesis are seen. A study of the interstitial tissue reveals that during the months when spermatogenesis is actively proceeding, the tissue is meagre, while during the winter months it increases in volume and is more conspicuous. I have assured myself regarding the inverse development of interstitial and sex cells and believe that the two are negatively correlated in this manner.

Figures 1 and 2 illustrate the two conditions. In the active testis, the interstitial tissue occupies the triangular areas between the roughly hexagonal locules and also similar areas in the periphery of the testis. The septum between the locules is a thin membranous partition with elongated stromal cells and practically no interstitial cells, which are confined almost exclusively to the corners of the locule. The locules are therefore largest in this condition and are filled with cells in different stages of spermatogenesis. Just the opposite kind of picture is offered by the testis in winter. Fig. 2 illustrates a part of the section of the winter testis. The locules are smaller, having shrunk in size. The septa between the locules are much thicker and are seen to be filled with interstitial tissue. The sex cells are few or are totally wanting and the whole testis presents the picture of a resting condition. The interstitial cells are grouped together in the form of distinct nests separated from one another by connective tissue and numerous such nests of interstitial tissue are seen throughout the testis between the locules. The contrast between the active and resting testis in regard to the quantity of interstitial tissue is very clear and striking.

Ichthyophis therefore presents a condition similar to that described by Champy (1913) in *Rana esculenta* where the development of the sex cells and interstitial tissue is never parallel and really takes place at different periods in the year.

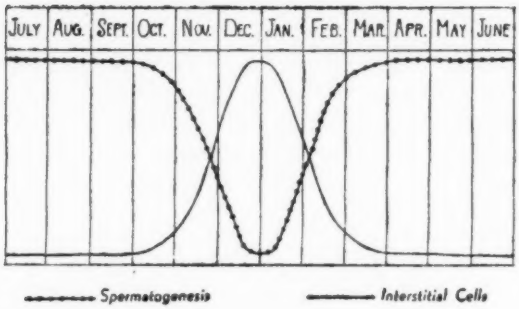

Diagram to show the relation between spermatogenesis and the development of interstitial tissue in the different months of the year.

Much has been written about the significance of interstitial tissue in the development of secondary sex characters, and even in Amphibia, two opinions obtain regarding this aspect of the problem. Champy (1913) has been unable to establish any relation between the two, while Aron (1921) and others clearly see a connection between the two. The case of *Ichthyophis* is very interesting. In this animal and in Apoda generally, external indications of sex, even during the breeding season, are wanting, and further, if the interstitial tissue is to be correlated with the development of external sex features, these characters must evidently be developed in winter when the interstitial tissue is at its highest and most abundant development. The absence of the development of any apparent secondary sexual characters at any period in the life of the animal goes to show that the interstitial tissue is not necessarily correlated with the development of secondary sexual characters.

It is now an established fact that in the majority of animals, the quantity of interstitial tissue varies in different seasons of the year. But it is a far more difficult matter to account for this variation in the tissue, and the exact manner in which the increase in the tissue at certain seasons of the year is brought about is not clear. From an examination of the literature on the subject it would appear that the increase in interstitial tissue is due to one or both factors: (1) Cell multiplication, involving the division, either by the amitotic or mitotic method, of the existing cells, thereby increasing their number. Direct amitotic division has been reported in interstitial cells by von Bardeleben (1897) in *Homo*, and must be considered, even when present, as a very rare and probably exceptional phenomenon. Division of interstitial cells by mitosis is certainly more common and there is no doubt that some contribution to the increased number is made in this manner

[Von Hansemann (1896); Rienke (1896); Von Lenhossek (1897)] in the human species. (2) A second method of increase in interstitial tissue has also been reported by a number of workers and takes the form of a transformation of stromal cells of the ordinary connective tissue type into typical interstitial cells [Mazetti (1911) in *Rana*; Rasmussen (1917) in *Marmota*; Humphrey (1921) in *Necturus* and other urodeles].

In this connection a paper by Blount (1929) is of considerable interest. Working on the horned toad (*Phrynosoma solare*) he has determined the seasonal changes occurring in the interstitial tissue and their correlation with the structure of the testis. His conclusions are interesting. He draws a significant distinction between the volume of interstitial tissue and the number of interstitial cells. He finds the volume of the tissue greatest during the breeding season. The size of the individual cells of the tissue is also very large at this period. But soon after the breeding season, the volume of the tissue decreases while the number of the cells is increased. How this is done is not clear. Apart from the fact that the author has drawn a distinction between the volume of the interstitial tissue and the number of cells of the tissue, the problem of the increase in the number of cells (which, in this animal, takes place after breeding) remains the same. If the number of cells is minimal during the breeding season, the question will be asked, how is the number reduced during the breeding season? Blount does not subscribe to the view of an inter-transformation between interstitial and stromal cells and does not notice any evidences of such a change in his material.

Now, with reference to *Ichthyophis* the problem of the seasonal changes in the interstitial tissue may be examined. It has already been observed that two distinct conditions can be noticed in regard to the interstitial tissue in this amphibian and that the development of the sex cells and of interstitial tissue appears to be correlated in a negative manner. I have not been able to make any tests of the type Blount has made in *Phrynosoma* and must content myself with changes as evidenced by appearance and quantitative examination.

It must be mentioned here that none of the authors who have seen either amitotic or mitotic divisions in the interstitial tissue of the animals they have studied is quite convinced regarding these divisions constituting the sole method of increase of the interstitial tissue. Divisions of either kind are too seldom to be of any value in increasing the tissue. The authors themselves admit this fact. Further, conflicting evidences regarding the occurrence or otherwise of these divisions in the same animal as examined

by different investigators add to our difficulty in accepting this method of increase of the tissue.

In *Ichthyophis*, I have not seen enough evidence for the conclusion that either mitotic or amitotic divisions contribute to the increase of interstitial tissue. I have not observed direct division in the nuclei of these cells, nor have I seen bi- or multinucleate cells at any time. A few mitotic figures have been encountered (Fig. 3) but never in such numbers as to warrant the conclusion that they are responsible for the increase of the tissue. These rare mitotic figures do not occur with any regularity. They are found at all times of the year and I believe they have no significance in the increase of the interstitial tissue in *Ichthyophis*.

The second method of the increase of this tissue remains to be studied. Rasmussen (1917) and Humphrey (1921) are amongst those who have observed a periodic transformation of stromal cells into interstitial cells and later, a regression into stromal cells. The increase and decrease of tissue (which, in *Ichthyophis*, means also an increase and decrease in the number of cells) are therefore due, according to them, to this transformation and retransformation. It means that at the season when the interstitial tissue is at its maximum, few stromal cells are seen and that when the interstitial tissue is poorly developed, the stromal cells are large in number. This is not borne out by the conditions found in *Ichthyophis*. No such inverse correlation between stromal and interstitial cells can be established. On the other hand, it is noticed that in the winter testis, correlated with the large quantity of interstitial tissue, large numbers of stromal cells also are found. I have no doubt that the stromal cells, like the interstitial cells, are larger in number and quantity in winter than during the rest of the year and that in fact, the increase in quantity applies as much to the stromal tissue as to the interstitial tissue.

One point of interest remains to be noted here. The disposition of interstitial cells in winter differs from that during the rest of the year. During the months when spermatogenesis is in progress in the testis, the cells occur as heaps in the triangular areas between the locules (Fig. 4). In the winter testis on the other hand, the cells occur in the form of groups or nests and each nest appears to be bound by a common membrane (Figs. 5 and 6). Isolated cells, either singly or in very small numbers do occur, but there is, on the whole, an orderliness in the grouping of the interstitial cells of the winter testis which is absent in the active condition. A similar arrangement has been noticed by Rasmussen (1917) in *Marmota*. He found this kind of grouping only in animals and in seasons where the interstitial

tissue showed maximum development, a finding which corresponds with mine in *Ichthyophis*, where in the winter testis, with the maximum development of interstitial tissue, this kind of grouping is seen. Rasmussen says " this would suggest that there is cell division and that each group of cells represents the daughter cells of a single parent cell ". But he has found no direct evidence of either mitotic or amitotic cell division, though occasional bi-nucleate cells are seen. My position is very similar to that of Rasmussen. While so far as appearance is concerned, the aforesaid grouping of cells suggests cell division, the actual evidence is insufficient to corroborate it and I also am obliged to leave the problem in this inconclusive condition.

Structure

In the active as well as resting conditions of the testis, the interstitial cells are the most conspicuous secondary cells of the organ. In the active testis they occur as isolated groups in the interstices between the locules or in the periphery and are far fewer in number than in the resting testis, an observation which is in conformity with that of Blount (1929) in *Phrynosoma*. The large size of the swollen capsules leaves very small triangular areas at the corners in which the interstitial cells are found. Very rarely do we find interstitial cells along the walls separating the adjacent locules. These walls are very thin and contain few cell elements of which the stromal cells are the most conspicuous. In regard to the size of the cells at the different seasons of the year, my observations confirm those of Blount. During seasons of greatest spermatogenetic activity the size of the individual interstitial cell is much larger than that during the season when the testis is at rest. In *Phrynosoma solare* Blount has also found that during the breeding season the greatest size in the interstitial cell is reached. In *Ichthyophis*, the size of the cells varies from 24 microns in the resting testis to 48 microns in the active testis.

The size of the nucleus, however, in the two conditions is apparently unvariable and I believe no difference in the size of the nucleus can be detected in the two seasons. Bi- and multi-nucleate cells observed in certain animals [Winiwarter (1912) in Man; Rasmussen (1917) in *Marmota*; Duesberg (1918) in the opossum] are not found in *Ichthyophis*. The extensive studies of Humphrey (1921) in a number of urodeles have not revealed any such cells in those Amphibia.

The nucleus of the interstitial cell is usually a deeply staining body. It is loaded with chromatin and stains far more intensely than the germ cell elements of the testis. Often it exhibits a definite indentation at one pole, generally at the pole in the neighbourhood of the centrosome. In

tissue fixed in Mann-Kopsch and Kolatschew fluids it is seen to be irregular in shape, evidently due to distortion. A number of nucleoli are present.

In the neighbourhood of the nucleus,—and if the nucleus is cup or kidney-shaped, in or near the concavity,—is the centrosome (Fig. 7). In Bouin preparations it assumes the form of a dense mass of protoplasm. In the centre of this dense cloud are the two centrioles which are always in the form of granules. Distinct radiations are seen in the centrosomal plasm.

In material fixed in osmic fluids, more particularly in Mann-Kopsch and Kolatschew material, the Golgi bodies are seen to occupy this region. They are in the form of a variable number of irregular curved rods of various sizes concentrated in the region of the centrosome (Fig. 8). A distinct Golgi region can therefore be distinguished in the neighbourhood of the nucleus in all preparations treated for Golgi bodies. Duesberg (1918) is probably the first to describe the Golgi apparatus in the interstitial cell but to my knowledge it has never before been described in the Amphibian interstitial cell. In his exhaustive and critical study of the interstitial tissue in the urodeles Humphrey (1921) makes no mention of this apparatus. I believe that the Golgi apparatus in the interstitial cell of *Ichthyophis* is in the form of a tangled network as depicted by Duesberg (1918) in the opossum.

Mitochondria in interstitial cells have long been known and have been described by a number of workers. Jordan (1911) was probably the first to study them. Winiwarter (1912) found them in Man and particular attention has been paid to them by Duesberg (1918) in the opossum and by Rasmussen (1918) in *Marmota*. So far as the Amphibia are concerned, the only work which makes mention of mitochondria in interstitial cells is that of Humphrey (1921) who has dealt with them at some length. In *Ichthyophis* they are quite conspicuous and are in the form of both grains and short rods. They may also occur as chainlike formations by fusion with one another, a very common feature. They lie scattered in the cytoplasm.

The presence of large quantities of lipoid material in the cytoplasm characterises the interstitial cells. Exceptions have so far been reported from the wild boar (Plato, 1896) and the Pig (Whitehead, 1908). Their presence has also been denied in the opossum by Jordan (1911) but the later studies of Duesberg (1918) on this animal seem to indicate that the lipoid content of interstitial cells tends to vary considerably, not only in different species but also in the same individual. The lipoid globules are of varying sizes in *Ichthyophis* and in a fully developed state they almost completely fill the cytoplasm, so that in osmic fixatives the whole cell looks quite black. In such cells the other cytoplasmic bodies and even the nucleus appear difficult to be

discerned. If the slides are treated with turpentine most of the fat globules become decolourised and the contents of the cell become clear. Such a cell is shown in Fig. 9. It is seen that the whole cytoplasm presents a bubbly appearance indicating the presence of fat globules practically crowding the cell space. The nucleus is clear and the Golgi bodies are seen at one pole of the nucleus in the form of a number of rods crowded together.

An examination of a cell where fat is being deposited reveals an interesting picture. In such a cell figured in 10, all the three cytoplasmic constituents, fat, Golgi bodies and mitochondria can be clearly seen. The Golgi bodies in the form of large irregular rods are conspicuous in the neighbourhood of the nucleus. The fat globules are mainly confined to this region and the mitochondria are scattered in the cytoplasm. The fat globules appear to arise as a result of the activity of the Golgi bodies. The origin of fat in the interstitial cell has not been accounted for by any worker satisfactorily. More than one kind of secretory material has been described but a satisfactory account of the origin of any of them has not been given. From my examination of the interstitial cells of the testis of *Ichthyophis* I am inclined to believe that the lipoid material which appears to be the only kind of secretion found in the interstitial tissue of this animal takes its origin as a result of the activity of the Golgi bodies.

From an examination of the cytoplasm of the interstitial cell it would appear that the deposition of fat in the cell is not in the nature of a transformation of the Golgi bodies into fat globules. For, even in the cell which is literally packed with fat globules, the Golgi bodies occur as rods in the neighbourhood of the nucleus (Fig. 9), crowded and pressed against the nuclear wall. This is only to be expected from the nature of things. The interstitial tissue is a permanent tissue of the testis and though its precise role is still in dispute, it is to be expected that it is of permanent usefulness to the animal. I believe that the deposition of fat is a continuous process in the interstitial cell, lasting over a long period, perhaps throughout the life of the animal. As such it is to be expected that the Golgi apparatus can only play the part of a structure that deposits fat, itself undergoing no change in the process. Secretion material other than fat has been reported by a number of workers, either intracellularly or intercellularly. Whitehead (1908) has seen them in a variety of animals. Rasmussen (1917) finds them in *Marmota*. Duesberg (1918) reports in the opossum networks inside the cell which he considers are in the nature of secretion products and which he believes are due to the transformation of mitochondria. This author finds similar material in the intercellular spaces and appears to think that this offers evidence of the secretory material finding its way into the circulation.

Humphrey (1921) has seen secretions in the interstitial cells of urodeles and believes that they are allied to and only slightly different from mitochondria.

The interstitial cells of the winter testis offer pictures which in many respects are different from those of the active testis. It has already been remarked that the construction of the interstitial tissue in the two conditions of the testis is different but the minute structure of the cells also is different. An interstitial cell from the winter testis is shown in Fig. 11. The nucleus is vesicular and the chromatin appears to be restricted to a few large bodies suspended in the vesicular nucleus. The cytoplasm reveals two distinct structures. First there is a cloud of granules which are usually grouped together in one place and at one pole of the nucleus. These granules are larger than the mitochondria encountered in the typical interstitial cell of the active testis and while they tend to group themselves in one place in the cell, a few may lie scattered in the cytoplasm. The second constituent of the cell takes the form of an amorphous lake occupying a large space in the cell. More than one such lake may be found in the cytoplasm of a cell. Both these structures take a deep black stain in hæmatoxylin, but while the former remain distinct and clearly separate from each other, the latter is a large dark body. It was at first thought that this different appearance of the interstitial cell of the winter testis was due to faulty fixation but examination of a number of sections prepared in a variety of methods corroborated the description of the cell given above. This was also confirmed by the appearance of the cell in Mann-Kopsch material where both the lake as well as the granules go black in osmic acid.

I have been unable conclusively to determine the real nature of these two bodies. Whitehead (1908), Rasmussen (1917), Duesberg (1917) and Humphrey (1921) have noticed in their material bodies larger than and different from mitochondria, but which are probably modified or distorted mitochondria. And it is possible that the laky material found in *Ichthyophis* might correspond to the amorphous secretion found by Duesberg in and outside the interstitial cells of the opossum. But I have not found intercellularly any secretion material corresponding with what Duesberg (1917) and Wagner (1923) have seen and am therefore unable to say what this material can be.

Summary

The interstitial tissue of the testis of *Ichthyophis glutinosus* varies in quantity and distribution in the active and resting conditions of the testis. In the former it is in the form of scattered groups of cells in the interstices of the locules, while in the latter it occurs as large number of nests of

cells between the locules. Fat is a characteristic cytoplasmic content of the interstitital cell and is believed to have arisen by the activity of the Golgi bodies. The significance of this fat or the general functional significance of the interstitial tissue of the testis is unknown.

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EXPLANATION OF FIGURES

FIG. 1. A part of a longitudinal section of *Ichthyophis glutinosus* during spermatogenesis. The locules are large and are separated by thin septa. Interstitial cells are found at the corners. × 75.

FIG. 2. A part of a longitudinal section of the testis of *Ichthyophis glutinosus* in winter when spermatogenesis is at rest. The septa are thicker and are filled with nests of interstitial cells. × 75.

FIG. 3. An interstitial cell in mitosis. Such mitoses are rare in *Ichthyophis glutinosus* and are considered of no value in the increase of the tissue. × 400.

FIG. 4. A corner of a locule of the active testis showing the disposition of interstitial tissue × 200.

FIG. 5. A portion of the interstitial tissue in the winter testis. The nest-like disposition of cells is clearly seen. × 200.

FIG. 6. A single nest of the same magnified. × 800.

FIG. 7. An interstitial cell showing the centrosome and the centrioles. Bouin. × 1800.

FIG. 8. The same: a Mann-Kopsch preparation showing the Golgi bodies. The vacuoles indicate the position of the lipoid globules which have been dissolved out. × 1800.

FIG. 9. An interstitial cell showing great vacuolation of its cytoplasm. The Golgi bodies are pressed close to the nucleus. Mann-Kopsch. × 1800.

FIG. 10. An interstitial cell showing the process of deposition of lipoid material. Its relation with the Golgi bodies is obvious. The small irregular rods and granules are the mitochondria. Mann-Kopsch. × 1800.

FIG. 11. An interstitial cell of the winter testis. The cytoplasm shows a number of granules and deeply staining masses of material. See text. Flemming without acetic. × 2400.

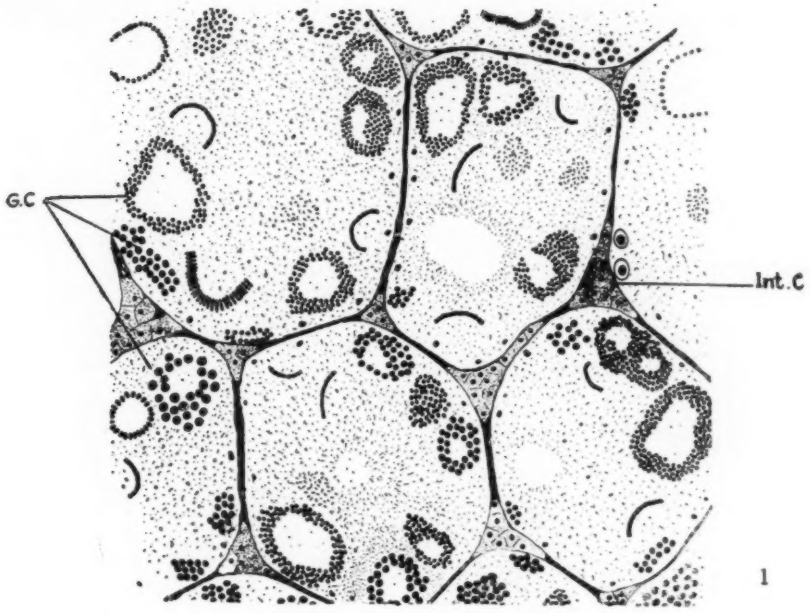
FIG. 12. Photomicrograph of a group of interstitial cells in active testis. × 375.

FIG. 13. A portion of the interstitial tissue of the winter testis showing the nest-like arrangement of cells. × 130.

FIG. 14. A Mann-Kopsch preparation of the interstitial tissue of active testis showing the vacuolated cytoplasm and the Golgi areas. × 575.

FIG. 15. A single interstitial cell showing the vacuolated cytoplasm, nucleus and the Golgi apparatus adjacent to the nucleus. Mann-Kopsch. × 550.

D. Collecting Duct; *G.* Golgi Apparatus: *G.C.* Germ Cells in various stages of spermatogenesis; *Int. C.* Interstitial Cells; *N.* Nucleus.



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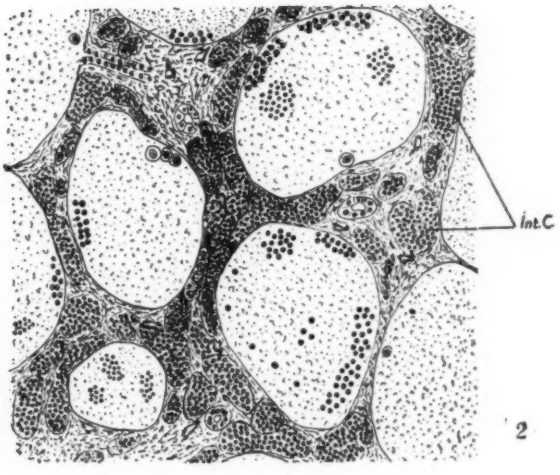

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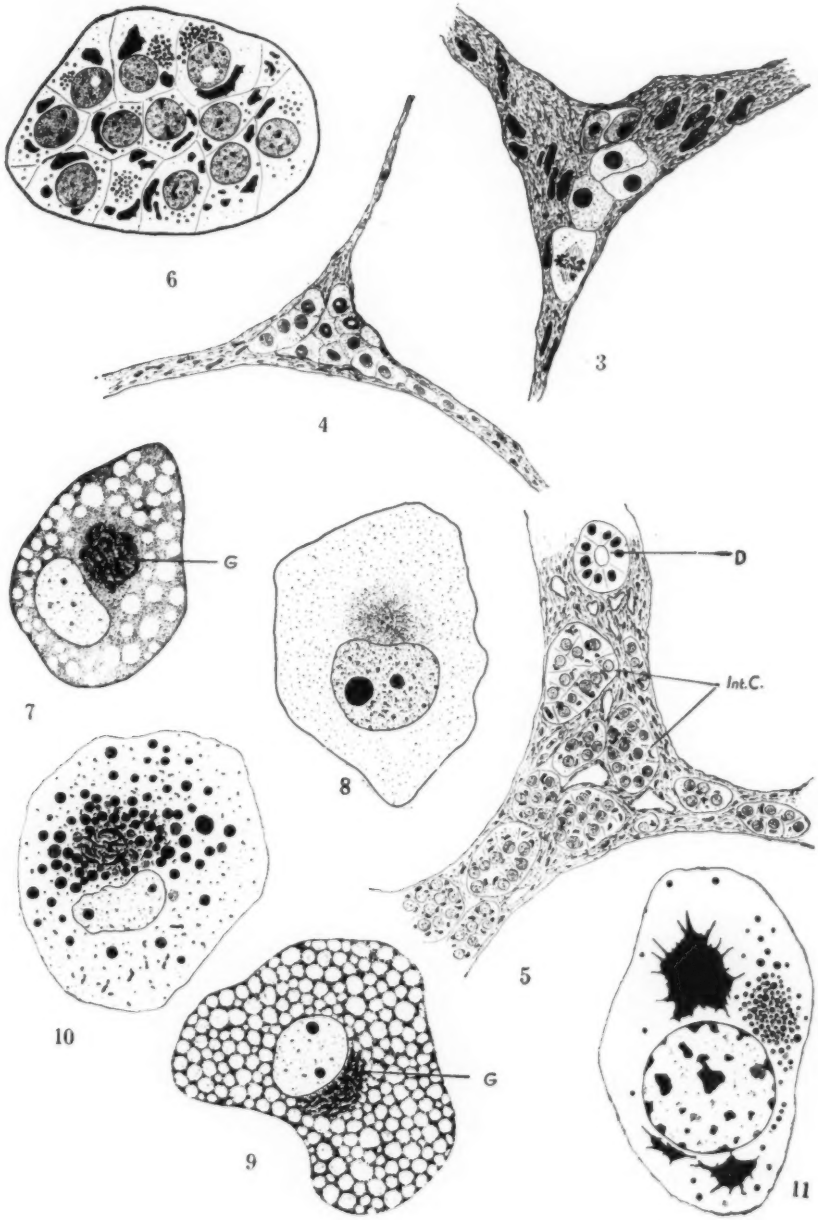
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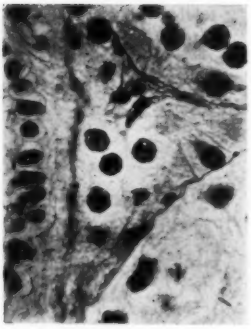

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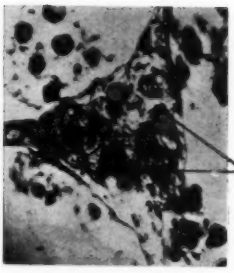

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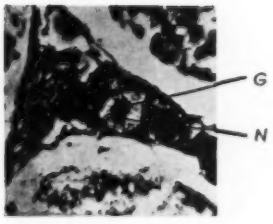

15

A STUDY OF THE FUNGAL ENDOPHYTE OF SOME *ANTHOCEROS ERECTUS* KASHYAP

By H. Chaudhuri and A. R. Quraishi
(*Botany Department, Panjab University, Lahore*)

Received November 5, 1940

The fungal endophytes of certain liverworts have been studied in detail from this laboratory. Chaudhuri and Rajaram (1925) described a case of symbiosis between the fungal endophyte and *Marchantia nepalensis*. Chaudhuri (1935) also isolated and made a comparative study of the fungal endophytes from *Petalophyllum indicum*, *Athalamia pinguis*, *Aitchisoniella himalayensis* and *Marchantia nepalensis*. In all these, the fungus was never absent from the thallus of the host tissue and the presence of the fungus produced no untoward effect on the host, though definite symbiosis has not been proved except in the last case. The fungal endophyte of *Anthoceros erectus*, however, is not universally found and when present the growth of the host is affected adversely. So in this case, the fungus behaves as a parasite, though it does not kill the host. It may be looked upon as a disease causing organism. The infected plants were collected from Mussoorie by Mr. P. N. Mehra in September 1935, and the authors, Chaudhuri and Quraishi, read a short note on a " New disease of *Anthoceros erectus* Kashyap " at the 24th Indian Science Congress Meeting in 1937.

The host plant.—Anthoceros erectus Kashyap is synonymous with *Anthoceros Butleri* Stephani. Kashyap (1929) found that there was practically no difference between the two species and he combined the two species under *Anthoceros erectus* Kashyap as Stephani established his species a year later than Kashyap.

This species is one of the very few annual liverworts and occurs in outer and Kumaon Himalayas from 5,000 to 8,000 feet, in Mussoorie, Kulu, Manali and also in Madras, Travancore and other places.

The infected plants were found growing on an exposed ridge with stony and gravelly soil. The diseased plants become stunted and develop a reddish tint, the healthy plants having bright green colour (Figs. 1 and 2).

The normal healthy plants grow upto 10 millimetres in diameter but measurements of twenty-five diseased specimens showed that none of them

exceeded 6 millimetres in diameter and the average plant measured only 4·5 millimetres. The sporogonia are formed but they do not grow to the normal size. The number of sporogonia produced on each thallus of *Anthoceros* was also reduced. More than three sporogonia on the diseased gametophyte were not observed, while the healthy plants produced normally five or even more sporogonia. Measurements of the length of the capsules exhibited the same phenomenon. Average length of the capsule in the diseased plants for twenty-five measurements was found to be only 10·2 mm., while the capsules of the healthy plants may be upto 30 mm. long. The viability of these spores could not be determined as neither these nor the bigger spores from the healthy plants germinated under local conditions.

The infected region of the plant shows hypertrophy—a tubercle-like structure is formed. This is reddish in tinge. Presence of fungal mycelium is seen in this region. *Nostoc* colonies which are universally present in these plants, become very conspicuous and bigger in the infected plants than in the normal plants (Fig. 5). It seems that the *Nostoc* plants, whatever their relation may be with the normal *Anthoceros* plants, try to take more than their due share, when the host plant itself is affected by the parasitic fungus.

Isolation of the fungus.—After the diseased plants were carefully washed in several changes of distilled water, they were treated with dilute mercuric chloride solution (1 in 1,000) for 30 minutes and washed again in several changes ot distilled water. Then the plants were placed between dry sterilised blotting papers to remove the water from the surface. Now small bits were cut aseptically from the swollen region and these bits were dipped in alcohol and flamed before planting on agar plates. In 4–5 days, fungal hyphæ were seen growing out. Bits of these hyphæ were removed and pure cultures were thus obtained.

Description of the fungus.—The morphology of the fungus was studied from the diseased material as well as from the cultures. The infected plants were fixed in formalin-acetic acid solution in 70% alcohol. Later paraffin blocks of the fixed material were prepared. Microtomic sections of these blocks, 12, 14 and 16 μ thick were cut and stained in light green, safranin, cotton blue, alcoholic eosin and erythrosin.

The fungus is confined within the tissue of the thallus. The fungus has not been found in the sporophyte. Examination of the sections, shows that the mycelium is intracellular. The hyphæ are found in all parts of the thallus but occur more generally in the basal region and conspicuously in some of the chambers. In the teased material and in some of the sections hyphæ were also observed in the rhizoids. This fact combined with the general

absence of hyphæ in the dorsal region of the gametophyte makes it probable that the hypha makes its entry into the plant tissue through the rhizoids. The hyphæ in the thallus tissue were of two kinds:—

(1) Branched, hyaline and thin-walled in which the septa were not distinct (Fig. 3). The diameter of these hyphæ is variable, ranging from $3{\cdot}5\,\mu$ to $5{\cdot}4\,\mu$.

(2) Branched septate hyphæ (Fig. 4) with thick contents and an average diameter of $6\,\mu$, occasionally forming knots in the cavities, or cells of the thallus. They often show considerable swellings at their tips. These hyphæ appear to be a later stage in the life of the fungus in the host tissue.

The effect of the fungus on the gametophyte is very well marked. Cells of healthy plants and of those parts of the infected thallus which escape the attention of the fungus possess a single large chloroplast and well-marked protoplasmic contents. In the infected region the bright green colour fades into reddish brown and generally the chloroplast and the protoplasmic contents suffer disintegration. It has also been noted how the infected plants remain dwarf and fail to produce healthy sporogonia of normal size. These facts leave little doubt regarding the parasitic nature of the infection.

Growth of the Fungal Endophyte in Culture

The fungal endophyte grows slowly in potato-glucose-agar. The hyphæ are hyaline when young but change to light brown when fully mature. Mature hyphæ are branched and septate and the cells are thick-walled. The breadth of the hyphæ varies from $2{\cdot}8\,\mu$ to $8{\cdot}5\,\mu$, although the hyphæ with the average diameter of $4{\cdot}8\,\mu$ are most common. The length of the cells varies greatly. The terminal cells have the greatest length and are correspondingly very narrow. Septa in the young hyphæ are very indistinct. The older hyphæ become closely septate and their cells are practically square shaped. Fully mature hyphæ form abundant chlamydospores which may be intercalary or lateral (Figs. 6, 7). Sometimes all the cells of the hyphæ become converted into chlamydospore-like structures. The dimensions of the chlamydospores are extremely variable, average being 7–$9\,\mu$.

Thick dark coloured sclerotia (Fig. 8) are formed in abundance in all media and are a prominent feature of the endophyte in culture media. The size of the sclerotia varies and an average for 25 counts in potato-glucose-agar media is $260\,\mu \times 310\,\mu$.

Examination of the sections of sclerotia shows only meshes of interwoven hyphæ with thick walls of black or dark brown colour. No conidia were

observed. The sclerotia on germination put out hyphæ which are at first very narrow, hyaline and without septa (Fig. 9). The septa appear later.

Growth characters of the endophyte were studied in different synthetic and other media containing vegetable extracts. Effects of different factors on the growth of the fungus were studied in cultures. Inoculations were done throughout by placing a single sclerotium from a four-weeks old culture of the endophyte (in potato-glucose-agar) in the centre of the Petri-dish containing the medium. Petri-dishes of uniform size were employed, and for each Petri-dish, a measured quantity of the medium, about 20 c.c. was poured. Measurements in millimetres were taken after every 24 hours. Two readings at right angles were taken for each Petri-dish.

Seven different media were used. The daily spread of the fungus and the cultural characteristics have been studied and are as follows:—

(1) The rate of daily spread varies widely in different media. It is least in Wort-agar, and in Nutrient agar the rate of linear growth is better only to that of Wort-agar. Growth is greatest in Purple lactose agar. In the remaining synthetic as well as in other media containing vegetable extracts, the growth in general is good. It flourishes well in sugary than in starchy media.

(2) In Wort-agar, where the rate of spread is least, luxuriant aerial growth is obtained, while in general the aerial growth is very moderate in all the other media and is least in Nutrient agar and in media containing no sugar.

(3) In all sugary media the colony is fairly compact, while it is loose in those lacking this ingredient. Sugary media are favourable for the formation of chlamydospores.

(4) Sclerotia are formed in all media, although their formation is much delayed in Nutrient agar and very few are formed in Czapek's medium.

(5) The size of the hyphæ varies in different media; the length of the cells and the dimensions of sclerotia also vary.

For studying the effect of different temperatures on growth, the rate of spread of the fungus on potato-glucose-agar was followed. All the Petri-dishes were inoculated at the same time and the experiment in each of the eight sets were run in triplicate. The data obtained are given below.

There was no growth at 37° C., even after 7 days, but the fungus was not dead: when subsequently the cultures were transferred to lower temperature growth resumed, though slowly. The optimum temperature was found to be 22° C. At 15° C. the rate of growth slowed down considerably, until

there was no growth at 6° C.; when the Petri-dishes incubated at 6° C. were transferred to 22° C., growth almost resumed at once.

The colony was loose and thin at lower temperatures, gradually becoming compact at higher temperatures. Aerial growth was scanty at low temperatures and only very moderate at higher ones. The size of the hyphæ and length of the cells also decreased with the rise of temperature. The brownish tinge of the hyphæ became more evident at higher temperatures. The chlamydospores were formed much earlier at higher temperature. The sclerotial formation was slow at lower temperature.

Effect of hydrogen-ion concentration was studied using eight grades of Czapek's medium with pH values ranging from less than 3·4 to more than pH 9·5.

The fungus could not grow in media with pH values less than 3·4 or greater than 9·5. While the growth was very slow in pH 3·4 or pH 9·5, maximum growth was obtained with pH 5·2 and the rate of spread gradually decreased in either direction. There was very little growth in alkaline media and sclerotial formation was scanty. In acid media the colony was more compact, there was abundant sclerotial formation and some aerial growth. The size of the hyphæ did not vary much. The chlamydospore formation was earlier in neutral media and did not take place in more alkaline or acidic media. The dimensions of the sclerotia was not very different.

Response to light was studied with three sets of potato-glucose-agar plates. One set was completely wrapped with black paper; the second set was left unwrapped. Both sets of Petri-dishes were incubated at the room temperature under a bell jar. A third set was left unwrapped in the dark room under a constant source of artificial light (sixty candle power) at a distance of eight feet. It was found that light had no perceptible influence on the spread of the fungus. The rate of spread and growth characters were same in all cases.

Though the fungus has been brown in various culture media under different conditions, apart from chlamydospore and sclerotia formation, no other spore form has been found. So, it has not been possible to name the fungus.

Summary

A fungal endophyte was noticed in the thallus of certain *Anthoceros erectus* from Mussoorie. These plants are stunted in growth and take up a reddish tinge, and the sporogonia on these plants are smaller in size. Healthy plants do not have any fungal endophyte. *Nostoc* colonies which are universally present, are more conspicuous in the infected plants. The endophyte

has been isolated and grown in culture in various media under different environmental conditions. Apart from formation of chlamydospores and sclerotia no other spore forms were produced.

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EXPLANATION OF PLATE XVI

FIG. 1.—Normal plant of *Anthoceros erectus*. × 3.

FIG. 2.—Infected plant of the above. × 3.

FIG. 3.—Part of a vertical section through the thallus of the infected plant showing branched hyphæ. Hyphæ unseptate. × 250.

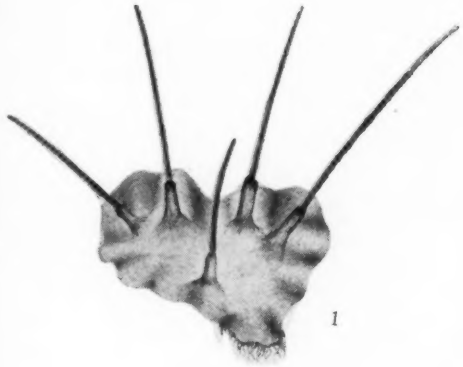
FIG. 4.—Part of thallus showing swollen branched hyphæ. Hyphæ septate. × 170.

FIG. 5.—Vertical section of the thallus through the *Nostoc* colony and showing the fungal endophyte. × 40.

FIGS. 6 & 7.—Chlamydospores on branched hyphæ. × 350.

FIG. 8.—Sclerotium with mature hyphæ. × 170.

FIG. 9.—Germination of sclerotium. × 170.



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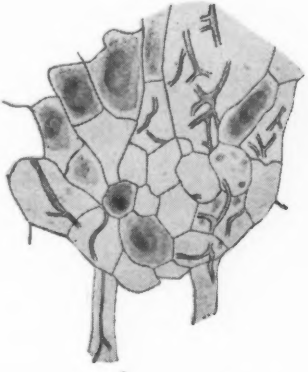

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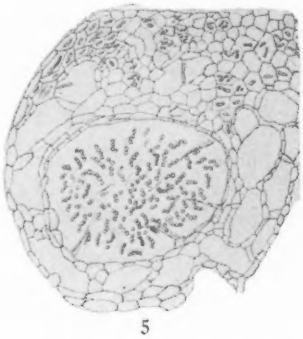

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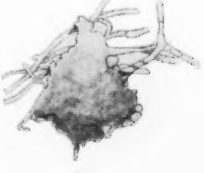

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A RAPID METHOD OF DETERMINING PEROXIDASE ACTIVITY

By M. Srinivasan, S. Ramaswamy and M. Sreenivasaya

(Department of Biochemistry, Indian Institute of Science, Bangalore)

Received February 12, 1941

It was reported previously (Srinivasan, 1936) that ascorbic acid oxidase in drumstick is accompanied by peroxidase. In subsequent experiments on the separation of these two enzymes, the need arose for a rapid method of determining peroxidase activity. We found that the method of Willstätter and Stoll (1917), involving, as it does, large volumes of reaction mixture and good quantities of ether, did not quite meet our experimental requirements. Search among other known methods indicated that the one based on the oxidation of benzidine to purpurobenzidine (Zirm, *et al.*, 1932) might prove simple and rapid. On actually trying out this method, however, it was found that the filtration of the dye prior to its dissolution in absolute alcohol, was tedious and time-consuming. It became obvious that the difficulty could be overcome by the use of a partially miscible and high-boiling solvent for extracting out the purpurobenzidine quantitatively. We observed that *n*-butyl alcohol was found to be an ideal solvent for the purpose. Based on this observation, the original method of Zirm, *et al.*, has been modified as described herein.

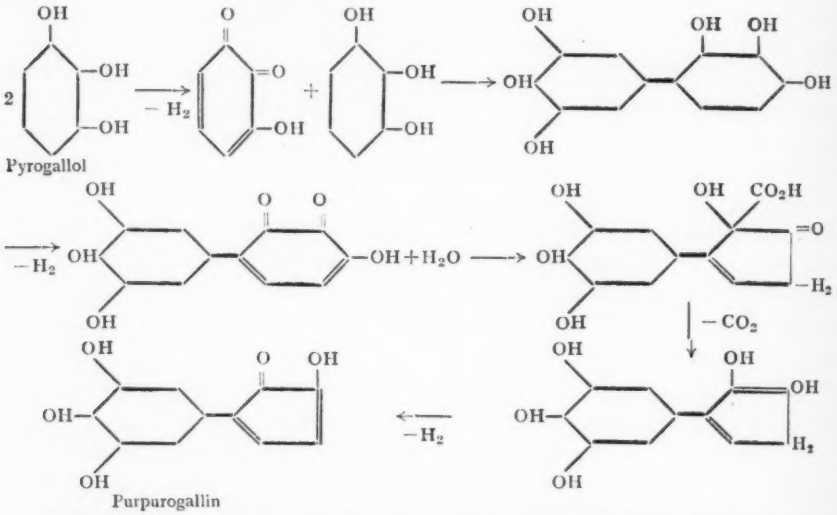
Different methods have been proposed from time to time for the quantitative determination of peroxidase. These methods are all based on the production of coloured compounds from one or the other of the following substrates in presence of H_2O_2 and the enzyme: Quinol (Bertrand, 1894); phenol (Bourquelot, 1896[1]); guaiacol (Bourquelot, 1896[2]); guaiaconic acid or guaiacum (Moore and Whitley, 1909); α-naphthol (Bourquelot, 1896[3]); benzidine (Schreiner, 1909); *p*-phenylene-diamine + α-naphthol (Vernon, 1911). Of these the guaiac reaction is unsatisfactory and unspecific for the determination of peroxidase, for, hæmoglobin, as well as hæmocyanins and many metallic chlorides give the reaction (Alsberg, 1908). One of the earliest methods for the quantitative determination of peroxidase is due to Bach and Chodat (1904). The method makes use of the liberation of iodine by peroxidase from acidified KI in presence of H_2O_2.

The method, now almost universally employed, is due to Willstätter and Stoll (1917). A solution of 5 g. of purest pyrogallol in 2 l. H_2O is

mixed with about 10 ml. of 5% H_2O_2 (exactly 50 mg.), regulated to 20° C. in a thermostat and then treated with 1–5 ml. of 5 mg. of the enzyme in 100–500 ml. H_2O. After exactly 5 minutes, the reaction is stopped by adding 50 ml. of dil. H_2SO_4 and the purpurogallin extracted with ether and estimated colorimetrically by comparison with a solution containing 100 mg. of the pure pigment in 1 l. ether. Elliot and Keilin (1934) found the estimation satisfactory and more convenient using one-fourth the quantities. Simply represented the reaction is:

$$2\,C_6H_6O_3 + 3\,O + H_2O = C_{11}H_8O_5 + CO_2 + 3\,H_2O.$$

Willstätter and Heiss (1923), however, found the mechanism of purpurogallin formation to be complex thus:—



Grassman comments on this method: ' Eigentümliche Schwankungen der enzymatischen Wirksamkeit, die bei reineren Peroxydasepräparaten beobachtet werden, lassen trotzdem die Sicherheit der quantitativen Methode zweifelhaft erscheinen. In der Tat scheint die Wahl einer so kompliziert verlaufenden Oxydationsreaktion nicht unbedenklich zu sein. Es ist mit der Möglichkeit zu rechnen ', " dass von den wechselnden Begleitstoffen der Peroxidase irgendwelche auf eines der sehr reaktionsfähigen Zwischenprodukte der Purpurogallinbildung einwirken und dadurch den quantitativen Verlauf der Reaktion stören ".

Therefore, Willstätter and Weber (1926) developed a simpler method which involves only one atom of oxygen without the formation of any

intermediate products. This method consists in the oxidation of leuco-base of malachite green in acetate buffer by peroxidase and H_2O_2. Quite inexplicably, this later method, however, does not appear to have found as much favour as the purpurogallin method.

The formation of indophenol from a mixture of *p*-phenylene-diamine and α-naphthol in citrate buffer at pH 4·5, is the basis of yet another quantitative determination of peroxidase, developed by Guthrie (1931) and later modified by Pack (1934).

Test for peroxidase activity using benzidine as substrate is due to Schreiner (1909). This qualitative reaction was developed into a quantitative method by Zirm, *et al.* (1932). Their method in brief is as follows:— A 1% solution of benzidine in acetate buffer is treated with an optimum concentration of H_2O_2 and the test solution of peroxidase. After an interval of 5 minutes, strong alkali (33%) is added, the precipitate consisting of purpurobenzidine and unreacted benzidine is dissovled in absolute alcohol and the colour of the resulting solution compared with a standard solution of the same pigment obtained by oxidation of benzidine by permanganate. The use of absolute alcohol, which the method enjoins, makes its imperative that the precipitate of the dye should be filtered off from the aqueous solution. This operation is by no means easy or quick, mainly due to the strong alkali that the reaction mixture contains. On the other hand, it was found that with the use of butyl alcohol, filtration could be avoided, the dye being quantitatively taken up from the reaction mixture by butyl alcohol. This constitutes the main improvement of the present method.

It should be emphasised that no attempt has been made here to evolve a method for the determination of the absolute activity of peroxidase in a given preparation; from that point of view, Willstätter's method is perhaps the best. Rather, the aim has been to evolve a method which would be simple and rapid and therefore useful for a series of comparative determinations of peroxidase activity of a preparation at various stages of its purification. From this standpoint, we found that the method of Zirm, *et al.*, but using butyl alcohol, was found eminently suitable.

We also thought it beside our present purpose to go into the question of the mechanism of the formation of purpurobenzidine. For the same reason, the correlation of purpurobenzidine number with that of purpurogallin has been omitted. We are aware of the more interesting question whether butyl alcohol cannot be employed with equal facility for extracting purpurogallin in Willstätter's method or the other dyes in the various

methods. The advantages of using butyl alcohol in the colorimetric methods are obvious.

Experimental

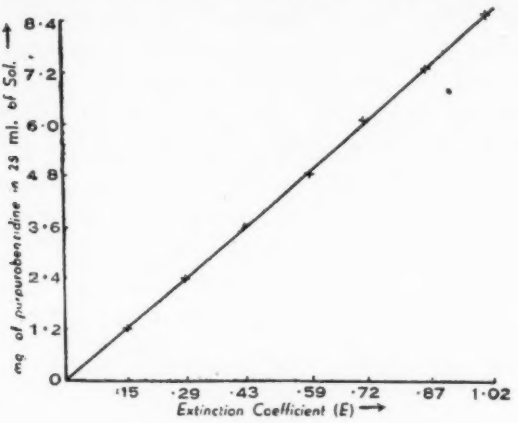
Preparation of the enzyme solution.—As source of enzyme, the fruit of *Sechium edule* (*cucurbitacæ* family) was chosen. The fresh vegetable is minced and pressed. The *press* juice, on centrifuging, yields a clear liquid. On half saturation with ammonium sulphate, a precipitate separates which is recovered by centrifuging. The precipitate is dissolved in the minimum amount of water and the resulting solution formed our stock solution of enzyme.

Purpurobenzidine.—Pure benzidine (2 g.) is heated with a mixture of 100 ml. N sodium acetate solution and 50 ml. of glacial acetic acid. The solution is filtered and the filtrate made up to 200 ml. The final solution is dark yellow in colour and has a pH of 3·5 to 3·65. To 60 ml. of this stock solution is added 90 ml. of N/200 $KMnO_4$ in a separating funnel (500 ml.). The mixture is vigorously shaken for 20 minutes when the solution attains a purple colour. Now strong alkali (30% NaOH) is added to make the solution distinctly alkaline to thymol blue (*i.e.*, pH $>$ 10·0). The red dye which is precipitated is extracted with 100 ml. butyl alcohol. The extraction is complete and quantitative, as the aqueous layer becomes colourless after the process. The butyl alcohol layer, which is coloured orange red, is washed free of alkali, dried with anhydrous sodium sulphate and filtered. The concentration of purpurobenzidine in this stock solution is found by the determination of the total solids in a measured volume of the solution. The stock solution we prepared had a concentration of 2·4 mg. of purpurobenzidine per ml.

Standard graph.—Different aliquots of the dye solution standardised as above are diluted to 25 ml., with ethyl alcohol. The extinction coefficients of these different solutions are determined in a Pulfrich Photometer (Filter, S_{53} and Cell, 20·06 mm.). The control consists of butyl alcohol diluted with ethyl alcohol in concentrations corresponding to the experimental solution.

From a reference to this standard graph (Graph I), the quantity of dye formed by enzymatic oxidation in the subsequent experiments is computed.

Determination of peroxidase activity.—After repeated trials, the following conditions were found to be most suitable for the determination of the peroxidase activity: 2 ml. of benzidine acetate solution (pH 3·5–3·65), prepared as above, are mixed with 48 ml. distilled water and aliquots of the test enzyme solution (suitably diluted). To this mixture contained in a separating funnel 1 ml. H_2O_2 (1·8 mg.), which was found to be the optimum



GRAPH I

Purpurobenzidine vs. E

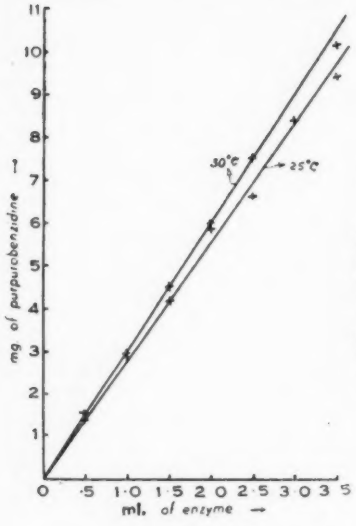
quantity (see Fig. 3), is added and well shaken. Exactly 5 minutes after the addition of H_2O_2, the reaction is arrested by the addition of 2 ml. of 25% NaOH. The solution is saturated with NaCl and shaken with 5 ml. butyl alcohol. After the separation of layers, the aqueous layer is drawn off and discarded. The butyl alcohol layer is run into a 25 ml. flask through a filter of cotton wool to retain insoluble impurities, if any. The separator is washed with ethyl alcohol and the washings passed through the original

TABLE I

Volume of enzyme solution (original stock solution diluted 50 times)	E (Observed)		Mg. of dye produced (calculated for the standard graph)	
	At 30° C. (thermostat)	At 25° C. (room)	At 30° C. (thermostat)	At 25° C. (room)
ml.				
0·5	0·18	0·17	1·49	1·40
1·0	0·36	0·35	2·98	2·88
1·5	0·54	0·51	4·48	4·21
2·0	0·73	0·71	6·00	5·89
2·5	0·90	0·81	7·46	6·60
3·0	1·03	1·02	8·54	8·41
3·5	1·23	1·15	10·20	9·46

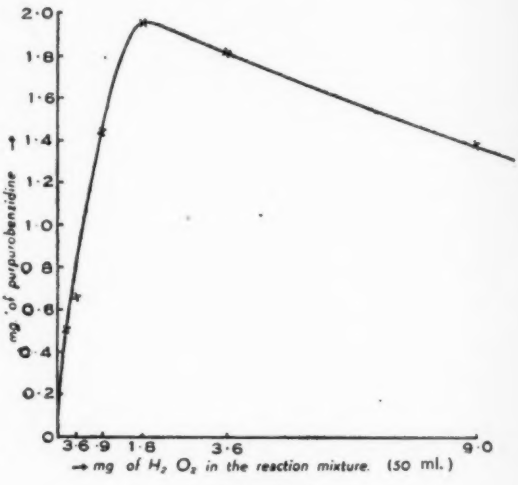
filter into the receiver. The contents are made up to 25 ml. with ethyl alcohol. A control is simultaneously run, but with the boiled enzyme. The extinction coefficients of the experimental and control solutions are read as before in the Pulfrich photometer. From these readings, the amount of dye is calculated from the standard graph.

The above table and Graph II represent the relation between enzyme action and dye formation. Under the conditions of the experiment, it is found, therefore, that the dye formed is strictly proportional to enzyme



GRAPH II

Enzyme Concentration vs. Purpuro-Benzidine Formation



GRAPH III

H_2O_2 Concentration vs. Enzyme Action (in terms of Purpurobenzidine)

concentration. Consequently, for the range of enzyme concentration studied, the quantity of purpurobenzidine which is determined, gives us an exact measure of peroxidase activity.

Summary

1. Butyl alcohol has been found to extract quantitatively purpurobenzidine formed from benzidine in acetic acid sodium acetate buffer (pH 3·5–3·65) by peroxidase and optimum concentration of H_2O_2.

2. This property has been made the basis of a quantitative determination (colorimetric) of peroxidase.

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AN ANALYSIS OF NORMAL ELECTROCARDIOGRAMS

Girls Aged 5 to 15 Years

By Rustom Jal Vakil, M.D. (Lond.), M.R.C.P. (Lond.), D.T.M. & H. (Lond.), F.R.F.P.S.G.

(Hon. Assistant Physician, King Edward Memorial Hospital; late Hon. Assistant Physician, J. J. Hospital; Tutor, Grant Medical College, Bombay and sometime First Assistant, Heart Hospital, Liverpool)

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An analysis of one hundred normal electrocardiograms from school-boys, ranging in age from 5 to 15 years, has already appeared in print in a previous issue of this journal. In the present issue, we present a study of fifty normal electrocardiograms from girls of school-age (5 to 15 years), the mode of study and selection of material being along similar lines. As a result of this study we are able to set forward normal electrocardiographic standards for Indian subjects of school-age.

The P Wave

The P wave or deflection represents the electrical activity of the auricular heart-muscle; hence the application of the designation "auricular wave" to this deflection.

Form of the P Wave.—It is customary to describe the P wave as "a smooth and rounded elevation"; such a description of the P wave has been handed down from author to author, without any enquiry into the true state of affairs. It was asserted in my previous communication, on the basis of one hundred normal electrocardiograms from boys of school-age, that the "pointed-form" of P wave is more commonly encountered than the "rounded form". In the present series of cases, the pointed form of P was about three times as common as the rounded form (the percentage frequencies being 53% and 17% respectively).

The greater frequency of the "pointed form" of P was obvious in all the standard leads (see Table 1).

Besides "pointed" and "rounded" forms, the P wave displays a variety of other appearances. Transition forms between the pointed and rounded varieties are met with. They were observed in 12% of records in Lead I and in 4% of records in Lead III. Flat or iso-electric P waves were seen in

Lead III in 16% of records. Inverted or negative P waves were noted in Lead III only, in 4% of records. Diphasic or bi-directional P waves were observed in Lead I in 2% of cases and in Lead III in 22%.

Befid P waves were seen in Lead I in 4% of records. In Lead II in 10% and in Lead III in 8%.

In 20% of records, there was no deflection of the P wave above the iso-electric line, in Lead III.

Notching of P waves.—Notching of the P wave is not confined to cases of auricular hypertrophy, as was once believed. It may occur even in normal records. In the present series, notching of P was observed in one or more leads in 22 records (*i.e.*, 44%) ; in no case was notching observed in all the three leads. Notching of P_1 was noted in 7 records (14%), of P_2 in 15 records (30%) and of P_3 in 7 records (14%). In 7 records (14%), there was notching of P in two leads ; notching of P_1 and P_2 in one record, of P_1 and P_3 in 2 records and of P_2 and P_3 in 4 records. In 15 records (30%), notching of P was confined to one lead only ; notching of P_1 in 4 cases, of P_2 in 10 cases and of P_3 only once.

Notching in P waves of large amplitude.—It is the general concensus of opinion that the association of large amplitude and notching in P waves should be considered pathological and suggestive of a diagnosis of mitral stenosis. In the present series, there was only one record with large and notched P waves (case 30 with notched P waves, 2·2 mm. in height). There was no evidence of mitral disease in this case. In the other cases of P wave notching, the amplitude of P ranged from 0·4 to 1·8 mm.

Relation of T to P Waves.—A relationship has been observed between the P and T waves of a given Lead, by various observers.

In the present series, it was noted that inverted P waves are always accompanied by inverted T waves. This rule, however, does not hold the other way about. With diphasic P waves (11 cases), the corresponding T waves were diphasic in 4 and inverted in 7 cases. With flat or iso-electric P waves (8 cases), the T waves were flat in 3, inverted in 4 and upright in one case.

Amplitude or Height of the P wave.—In my series of fifty normal school-girls, the average value of P for all Leads was 0·88 mm. The average value for Lead I was 0·79 mm., for Lead II 1·30 mm. and for Lead III 0·56 mm., the highest values being observed in Lead II.

Range.—In Lead I, the height of the P wave ranged from 0·0 to 1·4 mm., in Lead II, from 0·0 to 2·2 mm., and in Lead III, from –0·6 to 3·1 mm. The value of 3·1 mm. was only observed once in Lead III.

An analysis of the amplitude of P in the three leads is reproduced in Table 2.

Duration of P wave.—In the present series, the mean value of P wave duration for the three Leads was 0·081 sec. The average values for the individual Leads were:—

0·080 sec. for Lead I, 0·092 sec. for Lead II and 0·071 sec. for Lead III.

The duration of P ranged from 0·03 to 0·14 sec. in Lead I, from 0·06 to 0·14 sec. in Lead II and from 0·03 to 0·11 sec. in Lead III. The longest duration of P in my series was 0·14 sec. observed once in Lead I and once in Lead II. For further details, see Table 3.

P–Q Duration

This is measured from the end of P to the beginning of the Q wave.

In my series, the average value of P–Q for all Leads was 0·044 sec. The average values for the individual leads were: Lead I = 0·039 sec.; Lead II = 0·040 sec. ; Lead III = 0·052 sec.

Range.—In Lead I, P–Q duration varied from 0·01 to 0·07 sec., in Lead II from 0·00 to 0·09 sec., and in Lead III from 0·01 to 0·09 sec.

Maximum values for P–Q duration were found in Lead I in 22% of cases, in Lead II in 38% and in Lead III in 62%. A P–Q duration constant in the three Leads, was discovered in 4% of cases. Maximum values for P–Q were observed in two Leads in 14% of records; Leads I & II in 4% ; Leads II & III in 8% and Leads I & III in 2%.

Further particulars about P–Q duration are incorporated in Table 4.

P–R Interval

This is measured from the beginning of P to the beginning of QRS.

In this series, the value of P–R interval ranged from 0·08 to 0·20 sec., taking all Leads into consideration. The average values for the individual leads were:—

Lead I = 0·118 sec.; Lead II = 0·125 sec.; Lead III = 0·123 sec. The average value for all Leads was 0·122 sec. Tables 5 and 6 give our analysis of the P–R interval.

P–R interval in the Lead of Largest Measurement.—Maximum P–R interval values were observed 17 times in Lead I, 28 times in Lead II and 20 times in Lead III. The P–R interval was found equal in all Leads in three records. Maximum values for P–R were shared by two Leads on 9 occasions;

4 times in Leads I and II, 3 times in Leads II and III and twice in Leads I and III.

Values for the maximum P–R interval ranged from 0·10 to 0·20 sec., in the fifty records, the average value being 0·135 sec.

P–R Level or the Auricular T Wave

A mild degree of deflection (practically always in a downward direction) is a more or less constant feature of the interval between the end of P and beginning of QRS. It is due to auricular contraction and corresponds to the T wave of the ventricular complex.

In my series of 50 records, a downward deflection of the P–R interval was noted in all three Leads in 14 records (28%) and in two Leads in 22 records (44%) ; deflection of the P–R confined to one lead only was observed in 9 records (18%). In 5 records (10%), there was no deflection of P–R in any Lead.

Deflection of P–R was in a downward direction in all cases. It ranged from 0·0 to 2·1 mm. The average value of the P–R deflection for all Leads was 0·26 mm. In Lead I, P–R deflection ranged from 0·0 to 0·6 mm. with an average of 0·2 mm.; in Lead II, from 0·0 to 1·2 mm. with an average of 0·3 mm.; in Lead III, from 0·0 to 2·1 mm. with an average of 0·28 mm. (For further details see Table 7.)

Q R S Complex or Group

This complex is made up of three peaks or waves, Q, R and S.

Duration of Q R S Complex.—This is measured from the beginning of Q to the end of S. In my series of 50 normal school-girls, the duration of Q R S varied from 0·04 to 0·11 sec. The minimum value of 0·04 was seen in all Leads while the maximum value of 0·11 sec. was only observed once in Lead III.

The average value for Q R S, for all Leads, was 0·067 sec. Average figures for the three Leads, separately were:

Lead I = 0·067 sec. ; Lead II = 0·067 sec. ; Lead III = 0·068 sec.

(For further particulars, see Table 8.)

Duration of Q R S in the Lead of Largest Measurement.—The largest value of Q R S in any given record is usually taken as the correct measurement of Q R S.

The fifty records in my series were investigated from this point of view. Maximum values for Q R S were observed 21 times (42%) in Lead I, 20 times

(40%) in Lead II and 24 times (48%) in Lead III. In 4 records (8%) the same value of Q R S was shared by all Leads. Maximum values for Q R S were shared by two leads on 7 occasions (14%).

Values for maximum Q R S duration ranged from 0·05 sec. to 0·11 sec., in the fifty records with an average value of 0·073 sec.

(See Table 9 for further particulars.)

The Q Deflection or Wave

Incidence.—In my series of 50 cases, a Q wave observed in Lead I in 52% of cases, in Lead II in 60% and in Lead III in 70% of cases. In other words, in a series of 50 cases, Q was absent in Lead I 24 times, in Lead II 20 times and in Lead III 15 times. A Q wave was seen in all leads in 12 records (24%). In 20 records (40%), a Q wave was observed in two Leads, 4 times in Leads I and II, 11 times in Leads II and III and 5 times in Leads I and III. In 12 records (24%), the Q wave was confined to one Lead only, 4 times to Lead I, twice to Lead II and six times to Lead III. In 5 records (10%), there was no Q wave in any of the Leads.

Amplitude or height of Q Wave.—The height of Q varied from 0·0 to 3·4 mm. in the fifty records. The maximum value of 3·4 mm. was observed only once in Lead III. The average value of Q for all Leads was 0·57 mm. In Lead I, Q wave amplitude ranged from 0·0 to 3·0 mm. with an average of 0·48 mm.; in Lead II, from 0·0 to 2·1 mm. with an average of 0·38 mm. ; in Lead III, from 0·0 to 3·4 with an average of 0·86 mm.

(For further details see Table 10.)

The R Deflection or Wave

Amplitude.—In my series the height of R ranged from 1·0 to 14·6 mm. in the three Leads. The mean value for all Leads was 6·37 mm. In Lead I the amplitude of R varied from 1·8 to 11·2 mm. with an average of 6·0 mm.; in Lead II, it varied from 3·4 to 14·6 mm. with an average of 8·43 mm. in Lead III, it varied from 1·0 to 12·4 mm. with an average of 4·7 mm. The R wave amplitude values were highest in Lead II in the majority of records. In 40 records (80%), largest amplitudes of R were observed in Lead II, in 10 records (20%) in Lead I and in 1 record (2%) in Lead III. In Table 11, are recorded my analysis of R Wave amplitude.

Out of the 50 records in my series, there were 10 records (20%) with R waves in all Leads less than 7 mm. in height. The maximum deflection of R in these 10 records ranged from 4·1 to 7·0 mm., there was no evidence of heart disease in any of these cases. There were two records with the maximum R deflection under 5 mm. in height (4·1 and 4·6 mm.).

The S Deflection or Wave

Incidence.—An S wave was present in the majority of cases. It was observed in Lead I in 60% of records, in Lead II in 58% and in Lead III in 46%.

Height of the S Wave.—The amplitude of S ranged from 0·0 to 6·8 mm. in the fifty cases, the maximum value being observed once in Lead I. The average value for all cases was 0·79 mm. In Lead I, the height of S ranged from 0·0 to 6·8 mm. with an average of 1·01 mm. In Lead II, it ranged from 0·0 to 3·2 with an average of 0·77 mm. In Lead III, it ranged from 0·0 to 3·5 with an average of 0·58 mm.

In 7 records (14%), there was complete absence of the S Wave. S_1 and S_2 were absent in 5 records (10%) ; S_2 and S_3 were absent in 9 records (18%); S_1 and S_3 were absent in 2 records (4%).

An analysis of the S Wave is reproduced in Table 12.

Values over 6 mm. were observed in one record only, in Lead I.

Notching and Slurring of Q R S.—In this series of 50 cases, *notching* of R was not observed in Lead I or II in any of the records. Notching of R in Lead III was observed 8 times (16%). All R_3 waves with notching were of small amplitude (from 1·6 to 3·0 mm.) except in one case, where the amplitude of notched R_3 waves was 8·0 mm.

Definite notching of S was observed in one record only, in Lead III.

Hence, in normal records, notching of Q R S, if present, is usually confined to Lead III with small Q R S complexes.

" *Basal Slurring* " of R, that is thickening of the limb or limbs of R at its junction with the base-line, was observed in 22 of my records (44%). In no case was basal slurring of R observed in all three Leads. In 12 records (24%), it was observed in two Leads, once in Leads I and II, six times in Leads II and III and five times in Leads I and III.

In 10 records (20%), basal slurring of R was confined to one Lead only; twice to Lead I, twice to Lead II and six times to Lead III.

" *Apical Slurring* " of R, where thickening or splaying of the limb of R is not adjacent to the base-line, is a much more serious condition than basal slurring. In my series it was observed in 7 records (14%). In only one record was it observed in two Seads (case II with slurring in Leads I and III). It occurred 4 times in Lead I and 4 times in Lead III ; Apical Slurring of R was not observed in any record in Lead II.

R waves with apical slurring were of small amplitude (from 2·2 to 5·0 mm.) except in two cases, with amplitudes over 5 mm. (6·7 mm. and 6·8 mm. in cases 11 and 41 respectively).

In two records, there was combined notching and slurring of R waves.

Basal slurring of R was observed 11 times in the " upstroke " of R and 23 times in the "downstroke". Apical slurring of R was observed twice in the upstroke and 6 times in the downstroke.

The S–T Interval.—This is measured from the end of S to the beginning of T. Measurement of the S–T interval may be impossible in cases where T takes origin directly from the S wave. In the present series, the duration of the S–T interval in the three Leads varied from 0·01 to 0·16 sec. The minimum value of 0·01 sec., was only observed once, in Lead II ; the maximum value of 0·16 sec. was noted twice, once in Lead II and once in Lead III. The average value of S–T interval for the three Leads was 0·090 sec.

In Lead I, the S–T interval varied from 0·03 to 0·13 sec. with an average of 0·086 sec. ; in Lead II, it varied from 0·01 to 0·16, with an average of 0·088 sec. ; in Lead III, it varied from 0·03 to 0·16, with an average of 0·096 sec. An analysis of the S–T interval is reproduced in Table 13.

The S–T interval could not be measured in Lead I in 3 records, in Lead II in one record and in Lead III in 10 records.

In one record, duration of the S–T interval was identical in the three leads, the value being 0·09 sec. In 14 records (28%) maximum values for S–T were observed in Lead I. Of these 14 records, 6 showed values in lead II.

In 22 records (44%), maximum values for S–T were noted in Lead II. Of these, 6 showed similar values in Lead I and 6 in Lead III.

In 26 records (52%), maximum values for S–T were observed in Lead III. Of these, 6 showed similar values in Lead II.

From these figures, it will be noted that maximum values for S–T occur most frequently in Lead III.

Relation of the S–T segment to the base-line.—Deviation of the S–T segment from the P–R level was measured in the fifty records of my series. In Lead I, the S–T segment was at the level of the P–R segment in 23 records (46%), raised in 6 (12%) and depressed in 21 (42%) records. Depression of the S–T segment was much commoner than elevation (ratio of 3 : 5 to 1·0) in Lead I. In Lead II, the S–T segment was iso-electric in 19 (38%) raised in

7 (14%) and depressed in 24 (48%) records ; depression of S–T was 3 ·5 times as common as elevation. These findings are quite opposed to those of Hoskin and Jonescu, who find elevation of S–T to be much more common than depression, in Leads I and II.

In Lead III, the S–T segment was iso-electric in 23 (46%) raised in 17 (34%) and depressed in 10 (20%) cases. Elevation of S–T was more common than depression of S–T, in Lead III.

Deflection of S–T from the level of P–R seldom exceeds 1 mm. In my series, upward deviation of S–T ranged from 0 ·2 to 0 ·7 mm. Depression of S–T ranged from 0 ·2 to 1 ·4 in the fifty cases, the maximum value being attained only once in Lead I. Values over 1 mm. were encountered in only 2 records, the deviations being 1 ·4 mm. and 1 ·1 mm. respectively.

In one record the S–T segment was found depressed in all Leads. Elevation of S–T in all Leads was not found in a single record. In 6 records (12%), there was elevation of S–T in one Lead, depression of S–T in one Lead and isoelectric S–T in the remaining Lead. In 5 records (10%) S–T was raised in two Leads and depressed in one.

In 6 records (12%), S–T was depressed in two Leads and raised in one. 2 Records (4%) showed raised S–T in two Leads and iso-electric S–T in the remaining Lead. 10 Records (20%) showed depressed S–T in two Leads and iso-electric S–T in the remaining Lead. In 14 records (28%), deviation of S–T was confined to one Lead only; of these, 4 showed elevation and 10 showed depression of the S–T segment. In 6 records (12%), the S–T segment was iso-electric in all Leads.

S–T Duration

This is measured from the end of S to the end of T. In these series of fifty normal school-girls, the S–T duration ranged from 0 ·18 to 0 ·38 sec., in the three Leads. The minimum value of 0 ·18 sec., was found once in Lead III, the Maximum value of 0 ·38 sec. was noted once in Lead III. The average value of S–T duration for all Leads was 0 ·245 sec.

In Lead I, S–T duration ranged from 0 ·19 to 0 ·32 sec. with an average of 0 ·25 sec.: in Lead II, it ranged from 0 ·19 to 0 ·32 sec., with an average of 0 ·25 sec., in Lead III the range was 0 ·18 to 0 ·38 sec., and the average was 0 ·24 sec.

An analysis of the S–T duration is reproduced in Table 14.

The T Wave or Deflection.—The T wave forms the last of the ventricular deflections.

Direction of the Wave.—In the present series, the T Wave in Lead I was upright in all cases. In Lead II, the T was upright in all but three cases ; in case 18, it was diphasic, in case 20, it was flat and in case 24, it was inverted. In Lead III, T was upright or positive in 2 (4%), inverted or negative in 42 (84%), diphasic or bidirectional in 2 (4%) and flat or iso-electric in 4 (8%). In Lead III, therefore, the T wave was inverted in the great majority of cases.

Form of the Wave.—T waves can be classified into two broad groups according to the contour, *viz.*, the " pointed form " of T and the " rounded form " of T. In my series of fifty cases, the " pointed " or " peaked form " of T was encountered slightly more often than the " rounded form " (ratio of 12: 11).

In Lead I, the T wave was " pointed " in 36 records, " rounded " in 11 records and of the " mixed variety " (rounded and pointed) in 3 records. In Lead II, T was " Pointed " in 16, " rounded " in 30 and " mixed " in 3 records; in one record, T was iso-electric. In Lead III, T was " pointed " in 20, " rounded " in 24 and " mixed " in 1 record ; in 4 records, it was iso-electric and in 2, diphasic.

The proportion of " pointed " to " rounded " forms of T in the three Leads were as follows:

Lead I, 3·5: 1·0; Lead II, 1 : 2; Lead III, 5 : 6.

In 4 records, T was " pointed " or " peaked " in all Leads; in 5 records, it was " rounded" in all Leads.

Amplitude of T waves.—The amplitude or height of T ranged from–4·1 to 5·8 mm., in the three Leads. The mean value of T for all Leads was 1·17 mm.

In Lead I, the size of T ranged from 1·0 to 5·0 mm. with an average of 2·76 mm.; in Lead II, it ranged from 1·0 to 5·8 mm. with an average of 1·90 mm.; in Lead III, it ranged from −4·1 to +1·1 mm. with an average of −1·15 mm.

An analysis of T wave amplitude is reproduced in Table 15.

Size of T in the Lead of Largest Measurement.—In my series, the value of T in the Lead of largest measurement ranged from 1·4 to 5·8 mm. values of 2 mm. and under were observed eleven times (22%); values of 5·0 mm. and over were observed only twice (4%). Maximum amplitudes of T were observed in Lead I 41 times (82%), in Lead II 9 times (18%) and in Lead III only twice (4%).

(For further particulars see Table 16.)

Duration of T Wave.—In my series, the duration of T ranged from 0·08 to 0·26 sec., with an average value of 0·18 sec., for all Leads. In Lead I, duration of T ranged from 0·12 to 0·24 sec., with an average of 0·18 sec. In Lead II, it ranged from 0·14 to 0·26 sec., with an average of 0·19 sec. In Lead III, it ranged from 0·08 to 0·26 mm. with an average of 0·17 sec. The minimum value of 0·08 sec., for all Leads was attained only once in Lead III while the maximum value of 0·26 was attained twice, once in Lead II and once in Lead III.

An analysis of T wave duration is reproduced in Table 17.

Q–T Duration or Duration of the Ventricular Complex

This is measured from the beginning of Q R S to the end of T, in the Lead of largest measurement.

In my series of fifty normals, maximum values for Q–T were observed in Lead I on 21 occasions, in Lead II on 24 occasions and in Lead III on 13 occasions.

Maximum values of Q–T were shared by two Leads 9 times; 5 times in Leads I and II, once in Leads II and III and 3 times in Leads I and III.

The duration of Q–T ranged from 0·29 to 0·42 sec., in the fifty records, with an average of 0·344 sec. (see Table 18).

The Q–T Duration in Relation to the Heart-rate

A definite relationship is usually observed between the duration of Q–T and the rate of the heart, a gradual diminution of Q–T being observed with increasing rates of the heart (see Table 19).

The U Wave

The U wave or " the sixth wave of the electrocardiogram " was investigated in the fifty records of the present series. U waves were observed in one or more Leads in 20 records (40%). In no record were U waves observed in all the three Leads. They were noted in two Leads in 6 records (12%), five times in Leads I and II and once in Leads II and III. In 14 records (28%), U waves were confined to one Lead only, 4 times to Lead I, 7 times to Lead II and III times to Lead III.

Form of U Wave—

Two main forms of U wave were encountered (1) the " pointed form " and (2) the " rounded form ". In 14 records (28%), U waves were of the " rounded " variety only ; in 2 records (4%) U waves of both forms were

observed. In my series, the " rounded form " of U wave was 3·5 times as common as the " pointed form."

Incidence of U waves in Different Leads—

U waves were most frequently noted in Lead II. They were observed in Lead I in 9 records, in Lead II in 13 records and in Lead III in 4 records. The " pointed form " of U was observed in Lead I on two occasions only, in Lead II 4 times and in Lead III not once (see Table 20).

The amplitude of U ranged from 0·2 to 1·0 mm. in the fifty cases, with an average value of 0·36 mm. The average amplitude of the " pointed form " of U was about 1·7 times that of the " rounded form " (Table 21).

The *duration* of U ranged from 0·06 to 0·20 sec. with an average duration of 0·14 sec. The average duration of the " rounded form " was about 1·5 times that of the " pointed form " of U (Table 22).

Summary

(1) A study is presented of fifty normal electrocardiograms of schoolgirls, ranging in age from 5 to 15.

(2) Various deflections and " intervals " of the electrocardiogram are measured, analysed and tabulated, whenever possible.

The present investigation was made possible by a generous grant for the purposes of medical research from the Trustees of the late Sir Ratan Tata. My thanks are also due to the Trustees and Staff of the Parsee Girls' Schools' Association of Bombay for their kind co-operation in the collection of data for this work.

TABLE 1

Frequency of the Different Forms of P Wave in 50 Cases (Case Numbers)

Description of P Wave	Lead I	Lead II	Lead III
1. Pointed form	26	38	16
2. Rounded form	13	5	7
3. Pointed and rounded ..	6	0	2
4. Flat or iso-electric	2	2	8
5. Bifid form	2	5	4
6. Inverted form	0	0	2
7. Diphasic form	1	0	11

TABLE 2

Amplitude of the P Wave (Percentage Frequencies)*

Amplitude Range (in mm.)	Lead I	Lead II	Lead III
−1 to 0	0	0	4
0 to 1	84	24	44
1 to 2	14	62	16
2 to 3	0	10	2
3 to 4	0	0	2

* The amplitude of P could not be determined in 2% of cases in Lead I, in 4% in Lead II and in 32% in Lead III.

TABLE 3

Duration of the P Wave in 50 Cases (Percentage Frequencies)*

Duration of P Wave (in seconds)	Lead I	Lead II	Lead III
0·03	2	0	2
0·04	0	0	10
0·05	10	0	8
0·06	20	6	12
0·07	12	8	10
0·08	18	28	20
0·09	14	20	10
0·10	8	10	10
0·11	4	12	2
0·12	2	8	0
0·13	6	4	0
0·14	2	2	0

* The duration of the P Wave could not be determined in one case in Lead I, in one case in Lead II and in 8 cases in Lead III.

TABLE 4

Duration of P–Q in 50 Cases (Percentage)*

Duration of P–Q (in seconds)	Lead I	Lead II	Lead III
0·00	0	2	0
0·01	8	4	4
0·02	12	12	4
0·03	16	22	6
0·04	32	24	16
0·05	14	16	10
0·06	12	8	18
0·07	4	6	20
0·08	0	2	4
0·09	0	2	2

* P–Q duration could not be determined in Lead I once, in Lead II once and in Lead III 8 times.

TABLE 5

Duration of P–R Interval in 50 Cases (Percentages)*

Time Range (in seconds)	Lead I	Lead II	Lead III
0·08 to 0·10	26	16	12
0·10 to 0·12	44	34	36
0·12 to 0·14	22	32	24
0·14 to 0·16	2	12	10
0·16 to 0·18	4	0	2
0·18 to 0·20	0	4	0

* The P–R Interval could not be determined in Lead I once, in Lead II once and in Lead III 8 times.

TABLE 6

Duration of P–R Interval in 50 Cases (Percentages)*

P–R Duration (in seconds)	Lead I	Lead II	Lead III
0·08	4	4	2
0·09	4	0	0
0·10	18	12	10
0·11	18	6	10
0·12	26	28	26
0·13	14	20	8
0·14	8	12	16
0·15	0	8	6
0·16	2	4	4
0·17	0	0	2
0·18	4	0	0
0·19	0	0	0
0·20	0	4	0

* The P–R Interval could not be determined in Lead I once, in Lead II once and in Lead III 8 times.

TABLE 7
Deflection of P–R Level in 50 Cases (Percentages)*

Range of Deflection (in mm.)	Lead I	Lead II	Lead III
0·0 to 0·3	64	34	66
0·4 to 0·7	36	38	26
0·8 to 1·1	0	26	4
1·2 to 1·5	0	2	2
1·6 to 1·9	0	0	0
2·0 to 2·3	0	0	2

* Deflection of P–R, when observed, was in a downward direction in all cases.

TABLE 8
Duration of QRS Complex in 50 Cases (Percentages)

Duration of Q R S (in seconds)	Lead I	Lead II	Lead III
0·04	4	8	8
0·05	16	12	10
0·06	24	22	30
0·07	24	24	12
0·08	24	24	28
0·09	8	8	10
0·10	0	2	0
0·11	0	0	2

TABLE 9
Duration of QRS in the Lead of Largest Measurement (50 Records)

Range of QRS Duration (in seconds)	Lead I	Lead II	Lead III	For all Leads
0·04 to 0·06	5	3	5	8
0·07 to 0·08	13	12	17	30
0·09 to 0·10	3	5	2	11
0·11 to 0·12	0	0	1	1

TABLE 10
Q Wave Amplitude in 50 Cases (Percentages)

Q Wave Amplitude (Range) (in mm.)	Lead I	Lead II	Lead III
0·0 to 0·3	6	16	4
0·4 to 0·7	22	26	16
0·8 to 1·1	12	12	22
1·2 to 1·5	4	0	8
1·6 to 1·9	2	4	8
2·0 to 2·3	2	2	4
2·4 to 2·7	2	0	4
2·8 to 3·1	2	0	2
3·2 to 3·5	0	0	2
Absence of Q Wave	48	40	30

TABLE 11

Size of R Wave in 50 Cases (Percentages)

Size of R Wave (Range) (in mm.)	Lead I	Lead II	Lead III
0·0 to 2·9	6	0	30
3·0 to 5·9	50	14	40
6·0 to 8·9	28	46	26
9·0 to 11·9	16	30	2
12·0 to 15·0	0	10	2

TABLE 12

Size of S Wave in 50 Records (Percentages)

Size of S Wave (Range) (in mm.)	Lead I	Lead II	Lead III
0·0 to 1·0	66	72	78
1·1 to 2·0	16	20	14
2·1 to 3·0	12	6	6
3·1 to 4·0	4	2	2
4·1 to 5·0	0	0	0
5·1 to 6·0	0	0	0
6·1 to 7·0	2	0	0

TABLE 13

Duration of S–T Interval in 50 Cases (Percentages)*

Duration of S–T Interval (Range) (in seconds)	Lead I	Lead II	Lead III
0·00 to 0·02	2	2	0
0·02 to 0·04	12	4	4
0·04 to 0·06	4	18	4
0·06 to 0·08	24	22	20
0·08 to 0·10	28	26	26
0·10 to 0·12	22	18	14
0·12 to 0·14	2	6	10
0·14 to 0·16	0	2	2

* The S–T Interval could not be measured in Lead I in 3 records, in Lead II in one record and in Lead III in ten records.

TABLE 14

The S-T Duration in 50 Records (Percentages)*

S–T Duration (Range) (in seconds)	Lead I	Lead II	Lead III
0·16 to 0·18	0	0	2
0·19 to 0·21	12	6	16
0·22 to 0·24	28	28	32
0·25 to 0·27	40	32	20
0·28 to 0·30	16	22	10
0·31 to 0·33	2	4	0
0·34 to 0·36	0	0	0
0·37 to 0·39	0	0	2

* The S–T Duration could not be measured in one record in Lead I, in 4 records in Lead II and in 9 records in Lead III, owing to the iso-electric character of the T Waves.

TABLE 15

Size of T Wave in 50 Cases (Percentages)*

Size of T Wave (Range) (in mm.)	Lead I	Lead II	Lead III
−6·0 to −4·0	0	0	2
−4·0 to −2·0	0	0	8
−2·0 to 0·0	0	2	74
0·0 to 2·0	24	62	4
2·0 to 4·0	66	32	0
4·0 to 6·0	8	2	0
6·0 to 8·0	0	0	0

* The size of T could not be determined in Lead I once, in Lead II once, and in Lead III 6 times.

TABLE 16

Size of T in the Lead of Largest Measurement (Case Numbers)

Size of T (Range) (in mm.)	Lead I	Lead II	Lead III	For all Leads
0·0 to 2·0	8	3	0	11
2·0 to 4·0	30	5	1	35
4·0 to 6·0	3	1	1	4

TABLE 17

Duration of T Wave in 50 Records (Percentages)*

Duration of T (Range) (in seconds)	Lead I	Lead II	Lead III
0·08 to 0·10	0	0	2
0·10 to 0·12	6	0	2
0·12 to 0·14	0	8	18
0·14 to 0·16	14	22	22
0·16 to 0·18	28	20	22
0·18 to 0·20	34	20	16
0·20 to 0·22	10	12	4
0·22 to 0·24	4	10	4
0·24 to 0·26	0	2	2

* The duration of T could not be determined in two records in Lead I, in three records in Lead II and in four records in Lead III.

TABLE 18

The Q–T Duration (Maximum) in 50 Records

Lead	Number of records	Average value (in seconds)	Lowest value (in seconds)	Highest value (in seconds)
Lead I	21	0·341	0·300	0·360
Lead II	24	0·343	0·280	0·410
Lead III	13	0·374	0·290	0·420
For all Leads	50	0·344	0·290	0·420

TABLE 19

Q–T Duration in Relation to the Heart-Rate

Rate of Heart (per minute)	Number of cases	Average value (in seconds)	Minimum value (in seconds)	Maximum value (in seconds)
71 to 80	3	0·387	0·360	0·420
81 to 90	11	0·364	0·340	0·410
91 to 100	12	0·354	0·310	0·410
101 to 110	5	0·360	0·320	0·360
111 to 120	11	0·327	0·310	0·380
121 to 130	6	0·320	0·290	0·360
131 to 140	2	0·310	0·300	0·320

TABLE 20

Incidence of U Waves in the Three Standard Leads

Lead	Number of records with U Waves	Number of records with " rounded " U Waves	Number of records with " pointed " U Waves
Lead I	9	7	2
Lead II	13	9	4
Lead III	4	4	0

TABLE 21

Amplitude of the U Wave in 50 Records

	Average value (in mm.)	Minimum value (in mm.)	Maximum value (in mm.)
1. U Waves in general ..	0·36	0·20	1·00
2. " Pointed form " of U ..	0·52	0·40	1·00
3. " Rounded form " of U ..	0·32	0·20	0·60

TABLE 22

Duration of the U Wave in 50 Records

	Average value (in seconds)	Minimum value (in seconds)	Maximum value (in seconds)
1. U Waves in general ..	0·14	0·06	0·20
2. " Pointed form " of U ..	0·11	0·06	0·18
3. " Rounded form " of U ..	0·15	0·08	0·20

TABLE 23

A Summary of the Main Deflectors of the Electrocardiogram

Deflection	Feature investigated	Average value	Maximum value	Minimum value	Measurements in Lead of largest measurement
P Wave ..	Amplitude	0·88 mm.	3·1 mm.	0·0 in Leads I and II. −0·6 mm. in Lead III	
	Duration	0·081 sec.	0·14 sec.	0·03 sec.	
Q R S complex ..	Duration	0·067 sec.	0·11 sec.	0·04 sec.	Range: 0·05 to 0·11 sec. Average: 0·073 sec.
Q Wave ..	Amplitude	0·57 mm.	3·4 mm.	0·0 mm.	
R Wave ..	Amplitude	6·37 mm.	14·6 mm.	1·0 mm.	
S Wave ..	Amplitude	0·79 mm.	6·8 mm.	0·0 mm.	
T Wave ..	Amplitude	1·17 mm.	5·8 mm.	−4·1 mm.	Range: 1·4 to 5·8 mm.
	Duration	0·18 sec.	0·26 sec.	0·08 sec.	
U Wave ..	Amplitude	0·36 mm.	1·0 mm.	0·2 mm.	
	Duration	0·14 sec.	0·20 sec.	0·06 sec.	

TABLE 24

A Summary of the Main "Intervals" of the Electrocardiogram

Interval	Feature investigated	Average value	Maximum value	Minimum value	Measurements in Lead of largest measurement
P–Q Duration ..	Duration	0·044 sec.	0·09 sec.	0·00 sec.	
P–R Interval ..	Duration	0·122 sec.	0·20 sec.	0·08 sec.	Range: 0·10 to 0·20 sec. Average: 0·135 sec.
P–R Level ..	Deflection	0·26 mm.	2·1 mm.	0·00 mm.	
S–T Interval ..	Duration	0·090 sec.	0·16 sec.	0·01 sec.	
S–T Level ..	Deflection	..	0·7 mm. (upward) 1·4 mm. (downward)	0·2 mm. (upward) 0·2 mm. (downward)	
S–T Duration ..	Duration	0·245 sec.	0·38 sec.	0·18 sec.	
Q–T Duration ..	Duration	0·344 sec.	0·42 sec.	0·29 sec.	

CONTRIBUTIONS TO THE BIONOMICS, ANATOMY, REPRODUCTION AND DEVELOPMENT OF THE INDIAN HOUSE-GECKO, *HEMIDACTYLUS FLAVIVIRIDIS* RÜPPEL

Part II. The Problem of Locomotion

BY BENI CHARAN MAHENDRA

(*Department of Zoology, St. John's College, Agra*)

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1. Introduction

THE first part of the present series (Mahendra, 1936), besides giving a general introduction to the work, dealt with the systematic position of *Hemidactylus flaviviridis* Rüppel, its taxonomic history, geographical distribution, habits, habitat, reproductive phenomena and development. In the present part we take up one of the most fascinating problems connected with the creature—the mechanism of its locomotion.

The remarkable ability of the house-gecko to move with facility on walls, and even back downwards on ceilings, has attracted the attention of naturalists for a very long time and many authors have tried to elucidate the mechanism which makes these acrobatic feats possible. Whilst most of the workers have contributed substantially to our understanding of some aspect or other of the subject, none of the theories so far proposed fully explains the facts. We shall here first take stock of the phenomena of locomotion as gathered together by observation and experiment, then study the anatomy and histology of the foot to understand the structure of the locomotor apparatus, and finally discuss and evaluate the various theories propounded in this connection. In this way alone we hope to arrive at a true interpretation of the gecko's locomotion.

I am very grateful to Dr. Baini Prashad for the loan of volumes from the Library of the Indian Museum; to Professors K. C. Banerji and P. T. Chandi for going through the manuscript and giving me the advantage of their knowledge of Mechanics; and to Syed Muzammil Ali for assistance in the preparation of the illustrations.

2. *The Facts of Locomotion*

The observations on the ability of a gecko (*Hemidactylus flaviviridis* Rüppel) to move on and cling to vertical and tilted surfaces, as well as to the lower side of horizontal ones, can be divided into three categories :—

(*a*) Observations on intact living geckos.

(*b*) Observations on living geckos after amputation of all claws.

(*c*) Observations on freshly killed geckos.

In addition to these, attempts to remove all the subdigital lamellæ without either affecting the vitality of the animal or injuring its claw-bearing distal joint were also made, by amputation and by treatment with caustic potash solutions. It was found, however, that such operations are extremely painful and make the gecko either prostrate through nervous shock, or actually so crippled as to be useless for observations. It seems probable that the subdigital lamellæ are richly innervated and thus give a terrible shock to the creature in the process of removal.

Of the observations listed below, those marked with * are altogether new; those marked with †, old ones but with results differing from the ones previously recorded; those with ×, old ones with previously recorded results, confirmed by me ; and the unmarked ones, cited from literature but presumed to be correct.

(*a*) *Observations on living geckos with the foot intact.*

×(1) The house-gecko can move with facility on vertical and tilted rough surfaces.

×(2) It can move back downwards on ceilings, as well as on the lower side of horizontal rough surfaces. The size and weight of the individual seems to be an important factor in deciding this ability, the more bulky specimens being mostly incapable of such locomotion.

† (3) Many geckos of average size, though by no means all, can stick to and move on ordinary smooth glass surfaces, vertically placed, even when the latter are cleaned by xylol. In most cases, however, it is observed that they start slipping down as soon as they try to rest and that the locomotion is, on the whole, laboured. This observation differs from the first one of Hora (1924) in its result, as Hora records that " the gecko could *conveniently*[1] stick to a smooth vertical surface". Hora, however, does not definitely mention what surfaces he used for this experiment and whether he tried glass ones, cleaned by xylol. To uncleaned glass surfaces, the gecko can stick fairly conveniently.

† (4) The gecko cannot stick at all, upside down, to a clean smooth surface. Some individuals, however, can move in this manner on the lower side of a horizontally placed ordinary glass surface, if the latter is not perfectly clean.

× (5) It cannot move on or adhere to *wet* smooth surfaces, vertically placed. On such a surface, the gecko slips down even if the inclination is less than that of a right angle.

*(6) The gecko, while undergoing the process of sloughing, cannot stick to smooth surfaces, but after sloughing it can do so quite readily.

*(7) If a thin coating of glue is applied to the subdigital pads, the gecko finds it impossible to stick to smooth surfaces. When the glue is washed off, its power of adhering to smooth surfaces returns. This observation, as well as the previous one, indicates the importance of the cuticular hairs (*vide infra*) on the subdigital pads.

(8) " Considerable friction[2] was felt below the pads when the animal was drawn backwards by the hand, but when it was lifted vertically upwards or was drawn forwards very little adhesive power was apparent in these structures." (Hora, 1924.)

[1] The italics are mine.

[2] The word "resistance" should have been better, as the use of the word " friction " here is apt to prejudge the interpretation, besides being not strictly accurate.

(9) Weitlaner (1902) describes the following experiment on *Hemidactylus platyurus* : A living gecko was kept in a vacuum flask, in which a sheet of paper was fixed. It could climb moderately well on the smooth vertical glass surface inside, and very well on the paper fixed in the flask. As soon as the air was pumped out, it fell to the bottom of the flask, showing a convulsive curling inwards of the extremities, and gradually passed into a moribund condition. Further keeping it in the evacuated flask led to death. Since a dead gecko continues to adhere to the glass surface in spite of the vacuum (*vide infra*, Observation 18) it is clear that the living gecko falls down under such conditions not because of an inability of the locomotor mechanism but because of the agony of death.

(*b*) *Observations on living geckos after amputation of all claws.*

×(10). When all the claws have been removed, the ability of a gecko to stick to or move on surfaces is mainly conditioned by the roughness of the surface concerned. On very rough surfaces (*e.g.*, rough walls) it can either not stick at all or do so with much difficulty, finding locomotion almost impossible. On comparatively smooth surfaces (blotting-paper, ordinary paper, etc.) it can stick and even move to a fair extent, while on glass it does so as well as when the claws were intact. On perfectly smooth surfaces no adherence takes place.

×(11) The gecko with amputated claws finds it impossible to adhere to the lower side of horizontal surfaces, irrespective of whether the surface is rough, smooth or intermediate. This shows clearly that in the "upside down" manner of locomotion, the claws of the gecko are all important.

×(12) On wet glass surfaces placed vertically, the gecko without claws reacts as an intact one. It slips down, acquiring no foothold.

*(13) In order to find out whether there is any significant difference between the ability of an intact gecko and that of a clawless one to stick to smooth surfaces, the following experiment was tried: An ordinary-sized lizard which could stick to glass surfaces well was selected, and a thread tied round its waist just in front of the groin in order to support a fine string carrying at its free end a pan made of paper. After sticking the lizard to a vertical glass sheet, various weights were placed in the pan to determine how much weight the gecko could support without losing its foothold. It could carry 120 gm. in this way. The same lizard was similarly treated after amputation of claws and could support practically the same weight. In order to eliminate the effects of fatigue or nervous shock, in each case sufficient time was allowed before trying the experiment. The observations show that on comparatively smooth surfaces the claws are hardly of use for the gecko's adherence.

(*c*) *Observations on freshly killed geckos.*

Weitlaner's discovery (1902) that a freshly killed gecko, as well as even its extremities separated from the body, can adhere to various surfaces, opened a new method of approach to the subject and threw considerable light on the mechanism of a gecko's movement. Dellit (1934) confirmed and extended Weitlaner's experiments. The following observations are originally due to these authors.

×(14) The amputated extremity of a gecko can stick to smooth surfaces as well as the un-separated extremity of a living one.

(15) Even when toes of an amputated extremity are split in various ways and silken threads inserted in the splits so as to keep the air inside in communication with that on the outside, the foot sticks as well as the intact one. This observation indicates that the plantar surface of the foot does not act as a sucker, but that the subdigital lamellæ themselves are responsible for adherence.

(16) Weitlaner (1902) found that a single amputated extremity of a middle-sized *Hemidactylus platyurus*, when made to stick to a vertical sheet of paper, can support a weight of 80–90 gm. by a single thread. All the four extremities, stuck in this way, can support 400 gm.

(17) The amputated extremity can adhere only when pulled in the direction of its cut end, *i.e.*, in a line parallel to the foot musculature. When however, it has properly adhered, it can withstand, in the case of *Hemidactylus* (Weitlaner, 1902), a relatively strong pull even at right angles to this direction. Dellit (1934), who experimented on the gecko *Tarentola*, found it difficult to attach the amputated extremity and discovered that the slightest shaking served to dislodge it.

(18) Weitlaner (1902) found that a freshly amputated extremity of *Hemidactylus platyurus* continues to adhere to a vertically placed sheet of paper, even when a vacuum equal to $\frac{6}{7}$ of a perfect vacuum (*i.e.*, drawing 60–65 cm. mercury in a Baro-manometer) is created. Further, such an extremity can even support a weight in this condition.

(19) A lately-sloughed *Tarentola* (Dellit, 1934), when freshly killed, can adhere well to a window pane, but its amputated extremities do so with great difficulty, falling off at the slightest shaking. In a vacuum approximating to total evacuation (showing a pressure of 1 mm.), the attaching extremity invariably falls off, but the falling off may be as well due to the presence of a vacuum as to the shaking of the container by the action of the oil

air-pump. The various pressures at which the falling off takes place range from 5 to 90 mm.

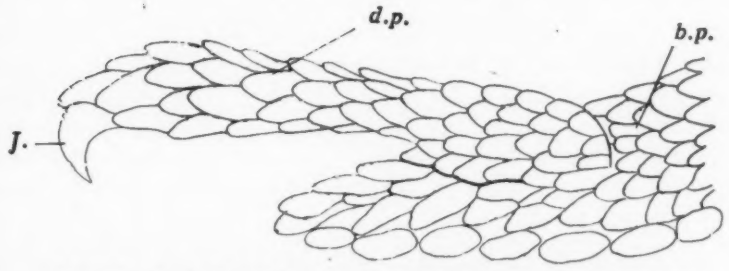
(20) Dellit (1934) killed a freshly sloughed *Tarentola* 7·8 cm. long and 12 gm. in weight and attached it to a sheet of paper, on which a 0·5 cm. scale was marked out. Keeping the position of the animal all along the same, he determined, by a stop-watch, its speed of sliding in normal air pressure, in a vacuum of 0·5 mm. with the motor still, and in a vacuum of 0·5 mm. with the motor working. The evacuation motor lay near the recipient so that the latter used to vibrate when the motor was in action. The gecko took the following times to pass through a distance of 5 cm.:

Air pressure normal	.. .. ..	160 seconds.
Vacuum of 0·5 mm. (motor still)	.. ..	135 ,,
Vacuum of 0·5 mm. (motor in action)	.. ..	29–40 ,,

By determining the palmar surface of the attached toes and calculating the pressure playing on it in vacuum conditions, Dellit concluded that even if a total vacuum were supposed to be present between the foot and the surface to which it is attached, the atmospheric pressure would be insufficient to support the weight of the animal (12 gm.). The great disparity between the time of sliding when the motor is still and that when it is in action seems to indicate that suction cannot account for the gecko's adherence.

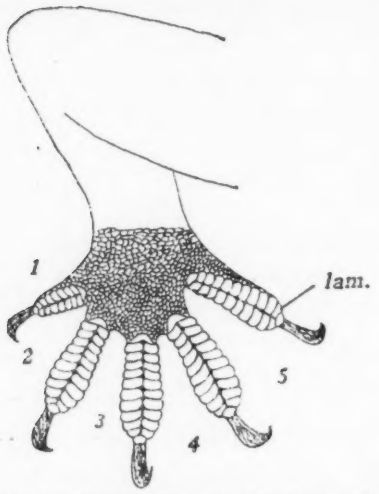
3. *The Structure of the Foot*

Each digit of the hands and the feet (Text-Fig. 1) can be divided into two distinct parts : a basal expanded portion bearing the subdigital lamellæ on



TEXT-FIG. 1. A digit of *Hemidactylus flaviviridis* Rüppel (Lateral View). *b.p.*, the basal portion; *d.p.*, the distal portion; *J.*, Claw.

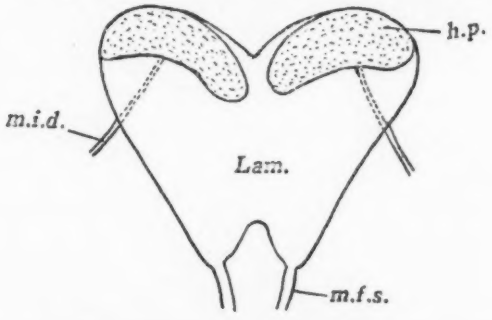
its ventral side (Text-Fig. 2), and a distal compressed portion terminating in a claw. The latter part arises rather angularly from the dorsal side of the



TEXT-FIG. 2. Ventral view of the foot of *Hemidactylus flaviviridis* Rüppel (× 4). 1, 2, 3, 4, 5, the five digits; *lam.*, subdigital lamellæ.

former a little behind the free extremity and is movable by means of special muscles. The distal part contains the last and the penultimate phalanges, while the basal one contains the remaining ones.

(*a*) *The subdigital lamellæ.*—The subdigital lamellæ in *Hemidactylus* (Text-Fig. 3) are heart-shaped with a median concavity directed towards the

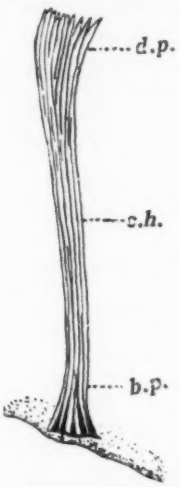


TEXT-FIG. 3. A single subdigital lamella of *Hemidactylus leschenaultii*. (After Dellit.) *h.p.*, " Haft-polster " or adhesion surface; *Lam.*, subdigital lamella; *m.f.s.*, tendon of Musculus flexor sublimis; *m.i.d.*, tendon of Musculi interossei dorsales

base of the digit. They are wider at the distal than at the proximal side and end proximally into two blunt processes on which the tendons of the *Musculus flexor sublimis* (Sanders) are inserted. The two lateral halves of the

lamellæ are very weakly united to each other in the middle line so that each half readily gets separated from the other in preparations (Dellit, 1934).

Each subdigital lamella, except the first and the last, bears two distinct, latreally disposed *adhesion surfaces* (' *Haft-polster* ' of German authors) near its free edge. The first and the last lamella each has only one such surface in view of the fact that it is not divided into halves. All the adhesion surfaces bear numerous microscopic brush-like processes of horny structure, generally supposed to be cuticular in origin (Cartier, 1872) but really intra-epithelial in their development as shown by Nicolas (1887). Each of these processes (Text-Fig. 4) is a hair-like growth, campact and rod-like at its proximal end,



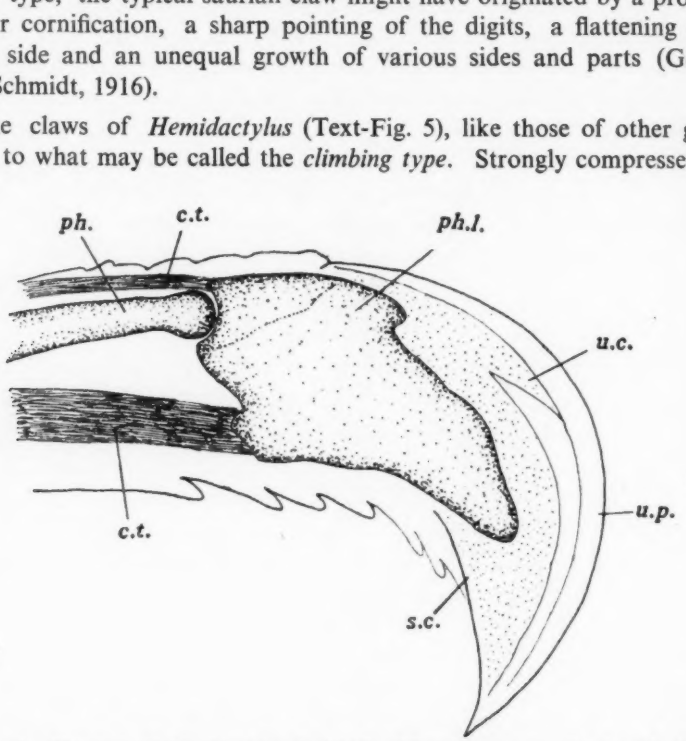
TEXT-FIG. 4. A cuticular process from a gecko's subdigital lamella. (After Dellit.) *b.p.*, basal part; *c.h.*, central part; *d.p.*, distal part.

split up at its distal extremity into a bunch of fibres and bent to one side towards their free edge. As Dellit (1934) points out, the length of these adhesion processes bears no relation to the size and weight of the individual gecko and is fairly uniform (84-118 μ) from one genus to another. Their individual fibres are most distinct from each other when the gecko has just emerged from its slough, but moisture, dirt and the passage of time tend to rob the fibres of their individuality and to weld them together.

(*b*) *The claws*.—About the claws of lizards, Wiedersheim (1875) made a short observation in *Phyllodactylus*; Boas (1894) described them in *Uromastix*, *Varanus* and *Iguana*, while Schmidt (1913) studied them in *Geckolepsis* and *Uroplatus*. Later, Schmidt (1916) scrutinised them carefully in most of the

saurian families and gave a detailed account even of their development. He traces their evolution from the conical cornifications found at the finger-tips of many Urodeles and thinks that the horny sheath equally developed all round (*e.g.*, in *Menobranchus*) was the most primitive condition. From such a type, the typical saurian claw might have originated by a process of stronger cornification, a sharp pointing of the digits, a flattening on the ventral side and an unequal growth of various sides and parts (Göppert, 1898; Schmidt, 1916).

The claws of *Hemidactylus* (Text-Fig. 5), like those of other geckos, belong to what may be called the *climbing type*. Strongly compressed from

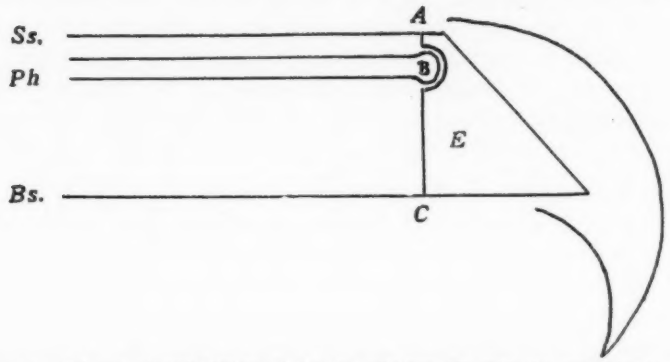


TEXT-FIG. 5. The claw of *Hemidactylus flaviviridis* Rüppel, from an alizarin-stained, cleared preparation.
c.t., contractor tendon; *e.t.*, extensor tendon; *ph.*, penultimate phalanx; *ph.l.*, last phalanx; *s.c.*, sole of the claw; *u.c.*, ungual canal; *u.p.*, ungual plate.

side to side, they are considerably high at their base and show an almost circular dorsal curvature from the base to the tip, the latter ending in a characteristic sharp and thin point. Each claw can be divided into two regions: a dorsolateral part, the *ungual plate*, formed of an upper and a lower piece enclosing between them a narrow tube, the *ungual canal*; and a ventral part, the *sole* of the claw. The two pieces of the ungual plate are strongly developed; the deep ungual canal, resembling a pointed cone in appearance, is a peculiarity of the family; the sole of the claw is much

shorter than the ungual plate; and the whole of the claw forms an encasement over the last phalanx of the digit, the ungual plate forming the dorsal and dorso-lateral parts of the covering and the sole of the claw the small ventral part.

Closely associated with the structure of the claw, the last phalanx of the digit shows a peculiar difference in its form from all the other phalanges preceding it. It is laterally compressed, broad dorso-ventrally and rather stout, tapers beak-like distally, and possesses an articular concavity for the rounded distal end of the penultimate phalanx on the dorsal half of its proximal border. The thin *extensor tendon* is attached towards its dorsal edge, and the strong *contractor tendon* on a large concavity on its proximal border ventral to the phalangeal joint. The displacement of the ball-and-socket joint between the last and the penultimate phalanx notably dorsalwards (Text-Fig. 6) has a



TEXT-FIG. 6. Scheme of articulation of the last phalanx (E) with the penultimate phalanx (Ph) in Gekkonidæ. (After Schmidt.)
B, distal end of penultimate phalanx articulated to the last phalanx; *Ss.*, extensor tendon attached at *A* to the last phalanx; *Bs.*, contractor tendon attached at *C* to the last phalanx.

very important effect (Schmidt, 1916). It makes the arm of the lever on which the contractor tendon plays several times larger than the arm on which the extensor tendon acts and thus serves to enhance the force of contraction or the bending of the claw ventralwards to a notable extent as compared to the extension or raising up. Had the joint (*fulcrum*) been placed symmetrically between the dorsal and the ventral surface, the force of extension or contraction of the claws would have depended entirely on the strength of the pull exerted by the respective muscles. As, however, it is actually situated asymmetrically far dorsalwards, the force of contraction increases, compared to that of extension, in the ratio in which the arm acted upon by it is greater than the other one. This has the advantage of strengthening the hold of the

claw-point on the locomotor surface. The unequally poised weight of the claw aids in the process.

Apart from this peculiarity, the other features of the claw may also be interpreted as adaptations for climbing. The delicate, needle-like tip can gain a hold in the minutest depression of the surface on which the gecko is moving. The strong curvature serves to distribute the pressure, which might otherwise have had to be supported by the slender distal end alone, to the entire structure and thus obviates the risk of the point breaking away in supporting the weight of the animal. The ball-and-socket type of joint permits free dorso-ventral movement of the claw-bearing phalanx on the penultimate phalanx. The division of each digit into a proximal (basal) and a distal part, connected to each other angularly, gives a greater latitude to the claw extremity for accommodation into the unevenness of the surface and makes it possible for the claw-point to be inserted *obliquely* downwards and backwards so as to withstand the force of gravity which acts perpendicular or parallel to the gecko's body but in no way in a straight opposite direction to the insertion of the claw-point. The fan-wise disposition of the five digits of the foot in five directions ensures that no two claws are inserted in exactly the same direction—a fact which leads to greater security of the hold.

(*c*) *The internal anatomy of the digit.*—Besides the facts mentioned above, we may note briefly two peculiarities about the internal anatomy of the digit, which may be regarded as relevant to the problem of a gecko's locomotion.

In the first place, the blood-vessels (Dellit, 1934) undergo a transformation into lacunæ in this region. In genera with undivided transverse lamellæ (*e.g.*, *Tarentola*, *Gecko*, *Ptychozoon*) the veins anastomose to form a single mesially situated cylindrical sinus of varying size, while in geckos with a bilateral arrangement of lamellæ (*Hemidactylus*) there are paired blood-sinuses situated dorsally.

Secondly, in *Hemidactylus* the *dorsal interossei muscles*, as well as the *M. flexor sublimis* end on the skin.

4. Previous Theories and their Discussion

The following five theories have so far been propounded to explain the phenomena of a gecko's locomotion.

(*a*) *The theory of adhesive secretions.*—The old notion that a gecko's feet secrete a sticky substance to adhere to the surface on which locomotion occurs has long been given up. There are three decisive objections against it. First, the most searching observations fail to establish the presence of such

an adhesive substance. Second, there are no glands in the feet which can secrete it (Cartier, 1872). Third, the supposed adhesive substance, in order to be effective, must be endowed with two qualities which are apparently contradictory to each other : on the one hand, it must be able to glue the feet *readily* and *firmly* to the locomotor surface ; on the other, it must be so obligingly yielding in its nature all along as to admit the separation of the feet from the surface without the slightest obstruction.

(*b*) *The pneumatic theory*.—Since Wagler (1830) first propounded the presence of minute vacua between a gecko's foot and the locomotor surface in order to explain the peculiarities of movement under consideration, the vacuum theory has gained a considerable popularity. Semper (1880), Simmermacher (1884), Graber (1886), Reuleaux (1900), Gadow (1901) and many other authors have implicitly accepted it. In its most popular form, it is believed that adherence takes place by the formation of interlamellar vacua. When the foot is pressed upon a flat surface, the soft and yielding plates are squeezed flat and the air in the spaces between them is driven out. Elevation of the centre of the foot is said to produce partial vacua between the plates, the feet acting as suckers on account of the play of the atmospheric pressure on their exposed surface.[3]

The untenability of the vacuum theory is shown by several facts. The absence of any arrangement to shut off the interlamellar grooves from the atmospheric air outside makes it difficult to imagine how the vacua are enclosed. Some genera like *Phyllodactylus* have a single pair of terminal lamellæ and are, therefore, devoid of the transverse interlamellar grooves. The fact that a freshly killed gecko can adhere as well as a live one militates against the view (Tandler, 1903) that the blood lacunæ in the toes, by diminution in size, produce suction. The insertion of silken threads in a variously split, amputated extremity and the latter's adherence in spite of it (Observation 15) indicates that the interlamellar space is not responsible for the phenomenon. Finally, the continuance of adherence even when a vacuum is produced around the amputated extremity or the entire gecko (Observations 18 and 19) rules the vacuum factor entirely out of consideration.

(*c*) *The electrical theory*.—H. Schmidt (1905) tries to explain a gecko's adherence as due to the electrification of the adhesion surfaces. He points out that the fine hair-like processes on the sub-digital lamellæ are not straight,

[3] Kunitzky (1903) rejects the vacuum theory and thinks that the pads act by close adpression, pressing out all air from between themselves and the surface. According to him, under these conditions they can stick by the sheer force of the atmospheric pressure alone. His theory also is untenable, because a dead gecko continues to stick when a vacuum is created around it.

ending in a point; but on account of a sharp bending, bear a small surface at their distal ends. According to him, the contact of thousands of these small surfaces with the surface to which the gecko adheres produces innumerable electric double-charges, which are responsible for adherence. This theory, having not been substantiated by any experimental evidence, can be regarded as only conjectural. The facts that a gecko can adhere as well on a 'conductor' as on a 'non-conductor', that experiments with a galvanometer fail to establish the presence of any electric charge, and that (as shown by Dellit, 1934) Röntgen and Radium rays do not cause the fall of an adhering gecko, are all against the Electrical Theory.

(*d*) *The adhesion theory*.—Haase (1900) regards *adhesion*[4], brought about by a close contact of the palmar surface of the digits with the locomotor surface, as the cause of a gecko's adherence. The closeness of contact, he thinks, is made possible first by the absence of bony scutes in the toes, and secondly by the presence of numerous lymph spaces in the lamellæ, which increase the plasticity of this region.

Of all the theories about a gecko's locomotion, the present one is the most difficult to prove or disprove definitely. The possible effect of the force of adhesion cannot be eliminated in any series of observations or experiments. However, if adhesion were the sole or even an important factor in a gecko's adherence, it would be difficult to understand why a clawless gecko can not only stick to, but even support a weight on, smooth *vertical* surfaces, while it falls off readily from tilted surfaces or from the underside of a horizontal surface (Observations 11 and 13). Why is it that the supposed *adhesion*, which is so very efficient on a vertical plane, fails to support even the body-weight in an " upside down" direction ? The two surfaces said to produce adhesion by contact apparently do not undergo any change and the force of gravity is quantitatively the same in both cases. The difference in the direction in which the force of gravity acts cannot be regarded as an important factor in deciding the efficiency or otherwise of *adhesion*, particularly as the latter must be regarded, according to the present view, a force capable of more than counterbalancing the quantitative value of gravity. Moreover, it is difficult to see how alterations in the direction of gravity relative to the axes of the body can have any effect on the strength of adhesion.

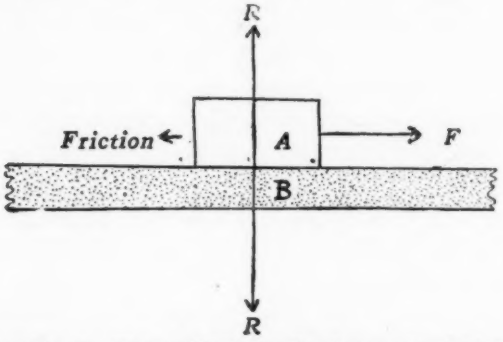
Another objection to the Adhesion Theory is similar to the third one, mentioned in connection with the Theory of Adhesive Secretions. How can adhesion be endowed with the two contradictory qualities of attaching the

[4] The phenomenon called into play by the attraction of molecules of different nature is called *dhesion* ; that by the attraction of molecules of the same nature, *cohesion*.

feet readily and firmly to the locomotor surface and at the same time, of not obstructing the locomotion in the least?

(*e*) *The friction theory*.—Hora (1923) regards the gecko's adherence to vertical surfaces as well as to the underside of horizontal ones, as due to the phenomenon of *friction*. "In the ridge-and-groove pattern on the digital discs and in the presence of innumerable hair-like excresecences found on the lamellæ," he finds "mere mechanical frictional devices which help to prevent the animal from slipping. The importance of the setose processes is apparent from the histological study of the pad, for we find several layers of cells whose only function is to produce these processes and to replace them as they are worn out."

Friction (Text-Fig. 7) between any two surfaces depends on two factors: the pressure between them, and the nature of the surfaces. It always acts



TEXT-FIG. 7. Diagram illustrating the phenomenon of friction.
If the block A, exerting a pressure R on the substratum B, is drawn by the force F, friction will come into play, acting in a direction opposite to that of the force F.

in a direction opposite to the one in which motion tends to take place and is directly proportional to the pressure.

The present theory, based on the force of *friction* for the elucidation of a gecko's locomotion, suffers from several serious drawbacks:

(1) Hora thinks that in the gecko's adherence to a vertical surface, "the weight of the animal itself causes pressure on the pads and makes them efficient". Actually, the body-weight, being due to the force of gravity, can exert no pressure in the direction imagined by Hora. It would act parallel to the vertical surface and tend to pull the lizard downwards.

If we imagine that the pressure is brought about by the voluntary contraction of the toe muscles, it is difficult to understand how a dead lizard can

exert it. In the absence of a pressure acting at right angles to the vertical surface, friction cannot come into play.

Dellit (1934) has tried to surmount this difficulty by supposing that the adhesion of thousands of microscopic lamellar processes to the locomotor surface replaces the pressure required and serves to bring friction into action. In a previous section I have already mentioned why the theory of adhesion is untenable, and those very objections apply to the acceptance of adhesion as an important factor here.

(2) Hora's attempt to explain the " upside down" mode of a gecko's adherence as due to friction are hardly convincing. He thinks that in such a posture, " when the limbs are stretched outwards, the weight of the body instead of pulling the pads directly downwards causes them to slip along the smooth surface for a short distance before exerting a vertical pressure on them. It is due to this fact that an animal can hang from a smooth surface when the limbs are stretched outwards. The pressing of the belly against the opposing surface is directly correlated with the position of the limbs, and is probably an additional advantage, since its scaly surface must help in increasing friction. Advantage may also be taken of atmospheric pressure by adpression."[5]

This sort of explanation, plausible at first sight, goes to pieces as soon as we examine the facts. The weight of the body, as pointed out by Dellit (1934), can be expected to exert only a negative pressure, tending thereby to separate the pads from the locomotor surface. The atmospheric pressure, mentioned by Hora in the foregoing quotation, is excluded on experimental grounds, and the fact that a gecko not only adheres to, but can also move on, the underside of a horizontal surface shows that the pressing of the belly against the opposite surface, is not of much importance. During movement, even in the back-downward posture, I have sometimes observed geckos keep their bodies separated from the locomotor surface to a fair extent. The limbs are stretched out only when the lizard is at rest, and that too not always:

Finally, experiments with numerous geckos (*Hemidactylus*) whose claws were amputated (Observation 11), invariably showed that the subdigital lamellæ have little, if any, share in the 'back-downward' mode of adherence,

[5] In a letter addressed to me (Aug. 7, 1935), Hora explains his view as follows: " The force of gravity pulls the lizard downwards and as its centre of gravity is somewhere about the middle, the great pull is there. The limbs are spread out at the sides, so that when the animal is pulled down from the centre, the pads slide against the ceiling and the frictional action of the pads is brought into play. The flattened body is also very helpful for this purpose. You must have noticed that the lizard is not as secure with its back downwards as when it climbs vertical walls." One wonders whether the author knows what is meant by the centre of gravity !

while the claws are all important.[6] This obviates the necessity of finding an explanation for such movements on the basis of friction. The gecko in such cases apparently makes use of its claws by inserting their sharp points into the extremely minute crevices in the ceiling and manages to secure a hold on the surface in this manner.

(3) The fact that the lamellæ offer resistance when the animal is drawn backwards, but do not do so when it is drawn forwards (Observation 8) is against the Friction Theory. If the efficiency of the lamellæ were due to friction, it would be immaterial in which direction they were pulled on the locomotor surface and thus they should have offered resistance equally in both cases. This observation, as well as observations on the amputated extremity (Observation 17) cannot be explained according to any interpretation *other* than the one, given by me later on.

(4) Observation 10 cannot be fully explained on the basis of the Friction Theory. While perhaps one could understand the gecko's inability to adhere to perfectly smooth surfaces and its ability to stick to comparatively smooth surfaces, one is at a loss to understand why very rough surfaces make adherence impossible.

5. *The Explanation*

The real explanation of the peculiarities of a gecko's locomotion is surprisingly simple.

As already mentioned, the movements on ceilings or the under side of horizontal surfaces are due to the action of the claws and not to the efficiency of the subdigital lamellæ (Observation 11). The extremely thin and sharp points of the claw can gain a firm grip into the minute irregularities of the surface and thus allow the animal to rest on, or move with fair facility, in a back-downward posture. This fully explains our observations 2, 4 and 11, of which the last cannot be explained by any other theory.

The action of the subdigital lamellæ is analogous to that of the claws. Each lamella has on its exposed surface a forest of microscopic cuticular processes, which are recurved at their tips. These processes get readily inserted, by adpression, into the microscopic irregularities of the locomotor surface and thereby secure a hold on it by a process of *interlocking*. Observations 8 and

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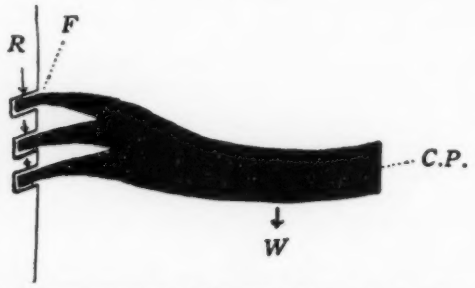
[6] Dellit (1934), however, reports that he could, with a great deal of difficulty, make a few of his claw-amputated lizards stick, back downwards, to the underside of a horizontal surface. This needs confirmation, as none of the numerous lizards experimented by me could do so, when their claws were removed. Dellit, it might be noted, worked mainly with *Tarentola*, a genus to which I have had no access so far. The explanation given by me for a gecko's locomotion can be applied even to such exceptional cases.

17, inexplicable on the basis of all other theories, definitely prove that the distal ends of the cuticular processes do engage into depressions of the locomotor surface. The cumulative effect of such innumerable holds must be really considerable (Observations 13 and 16). The slight bending exhibited by each cuticular process, considered together with the fan-wise disposition of the five digits of a foot in five different directions and with the difference in the directions of the feet themselves, indicates that the various groups of these processes catch at the irregularities of the surface from different angles, and this would naturally increase the intensity of the gecko's hold (*cf.* Observation 17).

The cuticular processes, according to this interpretation, function on the principle of the *lever* of the first type (Text-Fig. 8). The fulcrum (F) lies just



TEXT-FIG. 8. Schematic representation of the interlocking of a cuticular process (*C.P.*) with the depressions in the wall.
F., fulcrum; *R.*, reaction by the wall; *W.*, weight of the gecko's body.

behind the recurved distal tip of the cuticular process, the weight of the gecko's body plays on one side of it and this is balanced by the resistance, due to reaction, offered by the wall on the other side. Such a view is clearly distinct from the friction theory, although it must be mentioned that Coulomb (*Mem. de l'Inst.*, 1802, **3**, 246) tried to explain " boundary friction by assuming that the ' asperities ' or irregularities of the surfaces engage in one another".[7] Coulomb's theory has been long rejected by modern physicists, who believe that " friction is due to the molecular attraction one solid exerts on the other across the interface when the surfaces are perfectly clean, and to the inter-molecular attraction in the lubricant when the solid surfaces are covered by a film".[7]

[7] Quoted from Newman and Searle's *General Properties of Matter*, 1933, p. 220. *Cf.* also their remark, " There appears to be no justification for the view that static friction is produced by inequalities on a surface " (page 218).

The microscopic size of the cuticular processes determines their efficiency on locomotor surfaces of different grades of roughness. This explains the inability of a clawless gecko to adhere to very rough surfaces on the one hand and to perfectly smooth surfaces on the other, and its ability to adhere to moderately smooth surfaces, or (in other words) to surfaces showing an unevenness extremely minute (Observation 10). When the surface does not offer holds of the *requisite size*, the gecko naturally slips down (Observation 3).

The presence of water (Observations 5 and 12) prevents the lamellæ from gaining a grip on the locomotor surface probably on account of two reasons. First, it acts as a separating layer and does not allow the cuticular processes to come into intimate contact with the irregularities of the surface. Secondly, it deprives these processes of their individuality on acount of surface tension and tends to agglutinate them together. A similar effect is produced by the application of glue (Observation 7).

While the gecko is sloughing (Observation 6), the cuticular processes are covered by the dead skin ready to be cast off and thus they cannot get inserted into the locomotor surface. Hence the gecko's inability to adhere.

Finally, on surfaces too smooth for steady adherence, the gecko may often be able to move by gaining a temporary, though sufficiently long, foothold for progressing onwards. Friction, by retarding the speed of slipping down, may aid as a subsidiary factor in such locomotion.

6. *Summary*

After describing his own and other authors' observations on a gecko's movement and dealing with the relevant anatomical peculiarities of the locomotor apparatus, the author discusses the theories so far propounded in this connection. He finds that none of these theories (*viz.*, of adhesive secretions, of vacua, of electric charges, of adhesion and of friction) is tenable. In his opinion, the movement of geckos on the underside of horizontal surfaces is due to the sharp points of the claws taking a hold on the minute irregularities of the surface, while the action of the subdigital lamellæ is analogous to it. The cuticular processes on the latter get inserted by adpression into the microscopic depressions of the locomotor surface and thus maintain a hold on it by interlocking. They function on the principle of the lever of the first type.

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PHYSIOLOGICAL STUDIES ON THE WHEAT PLANT*

V. Diurnal Variations of Total Nitrogen and Amino-Acid Nitrogen in *Triticum vulgare*

BY SHRI RANJAN
AND
SANTOSH KUMAR BASU
(*From the Department of Botany, Allahabad University*)

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Introduction

IT is not our intention to give, in this small paper, a full historical resumé of work done in connexion with the diurnal variations of total nitrogen and amino-acid nitrogen, nevertheless it will be necessary, in view of the points discussed in this paper, to give a brief account of the more recent and important work done on the subject. Chibnall[2], working on *Phaseolus vulgaris*, established a strong probability that there are diurnal variations in the nitrogen contents of the leaves—the total nitrogen increasing by day and diminishing by night. He argues, therefore, that the nitrogen gets transported during the night. But he also says that the decomposition of proteins takes place both in the day and at night, but in the day the process is masked due to a greater synthesis of the proteins.

Maskell and Mason[5] also observed a diurnal variation of the total nitrogen in the cotton plant, so that the nitrogen increases by day and diminishes at night.

Regarding the work on the amino-acids Mc Kee[6] thinks that probably ammonia is to be regarded as the starting point in amino-acid synthesis and Dhar and Mukerjee[4] have found that amino-acids can be synthesized photochemically *in vitro*, using titanium dioxide as a catalyst. Blackman and Templeman[1] working on grasses found that in full daylight when ammonium sulphate and calcium nitrate is added, the total nitrogen, largely in the form of protein, increases. At low light intensities, although there is a rise in total nitrogen due to manuring, elaboration of protein is reduced. Muenscher's[8] work on chlorella shows that light is not a necessary factor in *in vivo* reactions. Nightingale[9] has shown that amino-acids are formed in the dark in the roots of *Narcissus* and *Asparagus*. Russell[12] working on the respiration

* Four other papers of this series have been published in the 1940–41 *Proceedings of the National Academy of Sciences*, India.

of *Elodea densa* finds that the amino-acid causes practically no increase in the rate of intake of oxygen.

The work on the diurnal variation of nitrogen, in the wheat plant, was undertaken in our laboratory to find out how far it was a photochemical process.

Material and Method

A. Material—

Samples of wheat plants (Pusa 52), growing in ordinary soil, were obtained from the botanical garden of the University. After removing the plants from the soil, they were immediately brought to the labotatory for analysis. For every experiment, samples were collected and analysed at 8 A.M., 12 Noon and 4 P.M.

B. Sampling—

(i) Leaf.—The technique employed in sampling leaves closely follows that of Maskell and Mason (1924). Each leaf sample consisted of about ten to fifteen mature and healthy leaves of practically the same age for every experiment.

(ii) Stem.—The technique followed in sampling the stems was the same as that of the leaves. Sheathing leaf bases and very small portions of stems had to be employed as experiments were started when the plants were very young. From a number of plants, 25 to 30 equally thick pieces, each of about half an inch in length, were selected for every experiment.

C. Method of Analysis—

(i) Leaf.—Five grams of fresh and healthy leaves were quickly weighed, cut into small pieces and put into briskly boiling distilled water, sufficient to cover the material. The leaves were boiled for about five minutes. It was then finely crushed with some purified sand in a hand mortar and boiled again. It was then filtered and the residue was washed several times with hot distilled water. The filtrate was concentrated to a fixed volume and analysed for amino-acids. The residue was analysed for total nitrogen. *The total nitrogen, therefore, represents the nitrogen of the non-soluble proteins only.*

(ii) Stem.—The procedure, for stem analysis, was the same as for the leaf.

D. Estimations—

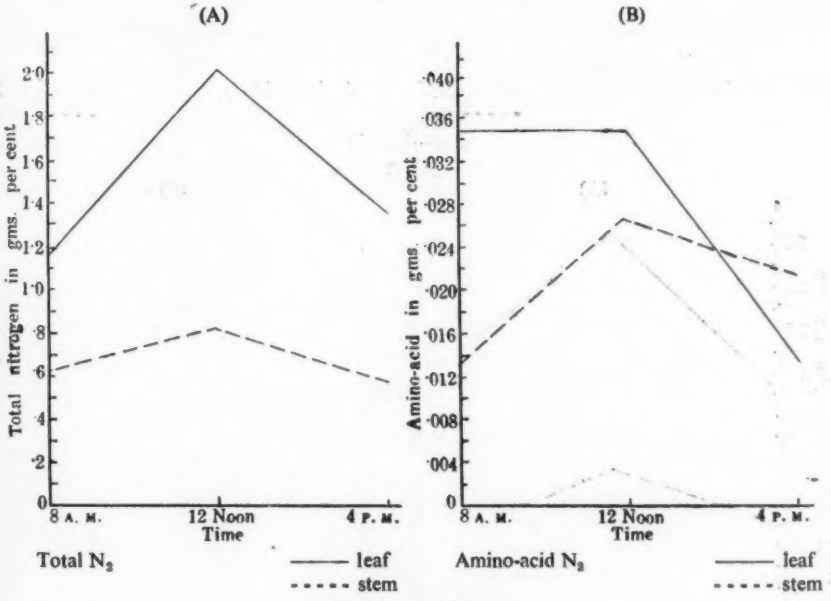
The amino-acid nitrogen was estimated by the Van Slyke's method and the total nitrogen by Kjeldahl's method using Nessler's reagent. The colour

of the solution was matched against a standard solution by the help of the Hellige colorimeter.

Experimental Results

Section I—

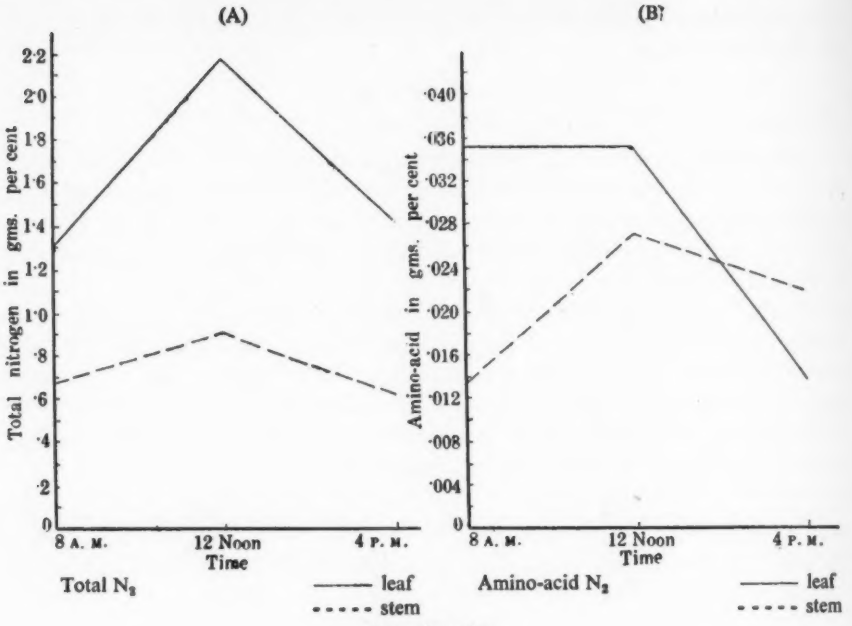
The diurnal variation of total nitrogen and amino-acid nitrogen in stems and leaves during the ontogenic drift of the wheat plant.—The experiments were started with wheat seedlings grown in sawdust, which were from 10–14 days old. The amounts of total nitrogen and amino-acid nitrogen in the stems and leaves are given in Fig. 1. The total nitrogen rises from about 1·2 in the leaf in the morning to nearly 2 gms. per 100 gms. of leaves at noon and thereafter it falls to a little over 1·3 gms. In the stem also the total nitrogen rises from 0·6 to 0·8 at noon and then it falls off to 0·6 gms. The amino-acid nitrogen of the leaf keeps steady at 0·035 till noon and thereafter it falls off to 0·013. The amino-acid of the stem rises from 0·013 in the morning to 0·027 at noon and then slightly declines off to 0·022.



Age—10 to 14 days

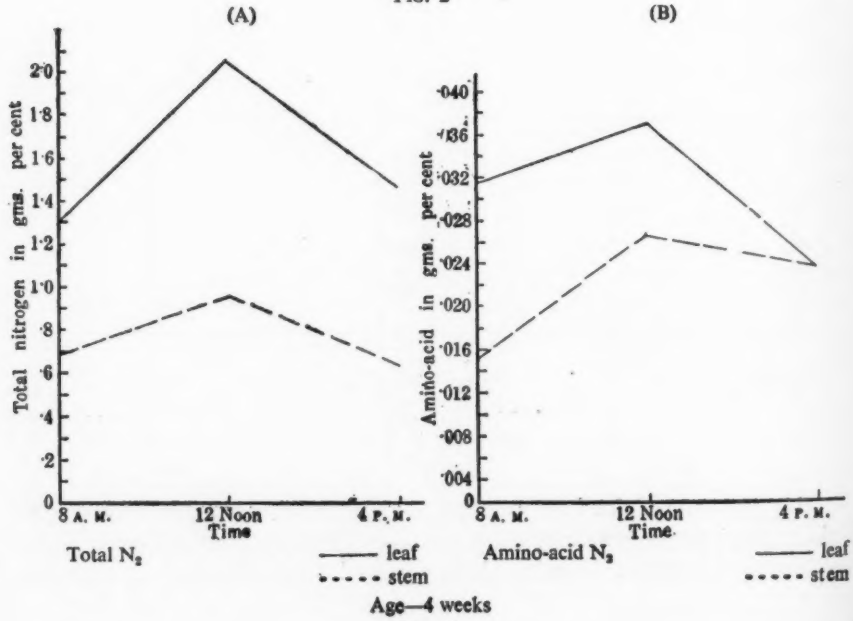
FIG. 1

This sequence of rise from morning to noon and then a fall in the evening, in the total nitrogen, is seen in the wheat plant throughout its ontogenic drift, *e.g.*, in plants 3 weeks, 4 weeks, 1½ months and 2 months old.



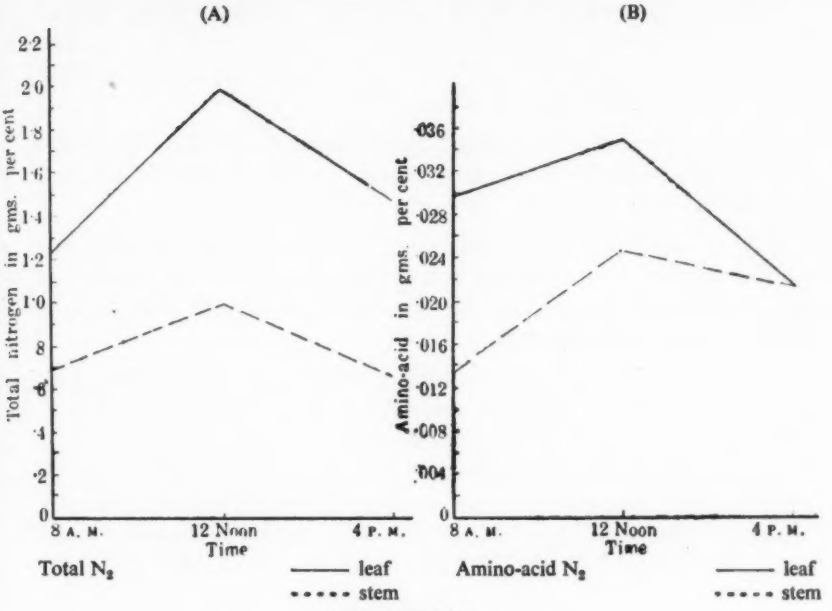
Age—3 weeks

FIG. 2



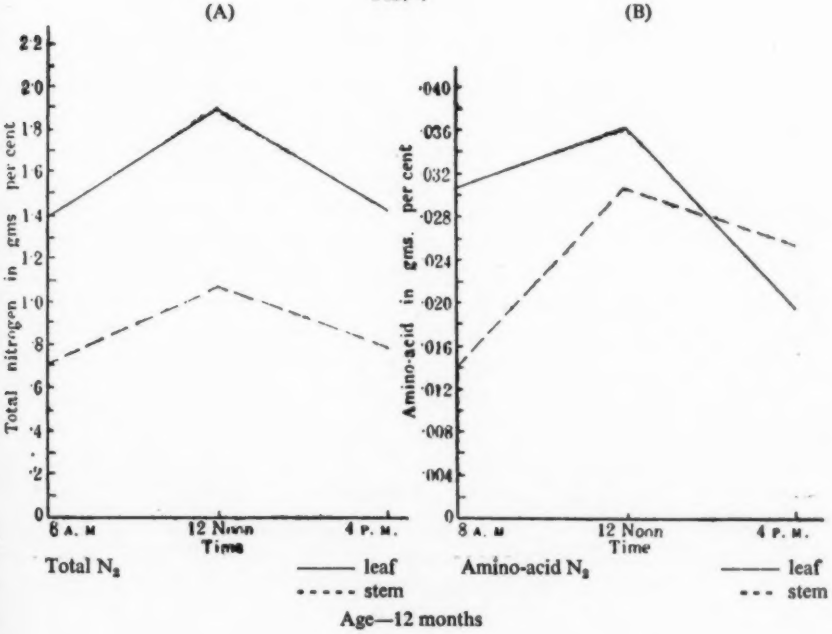
Age—4 weeks

FIG. 3



Age—1½ months

FIG. 4



Age—12 months

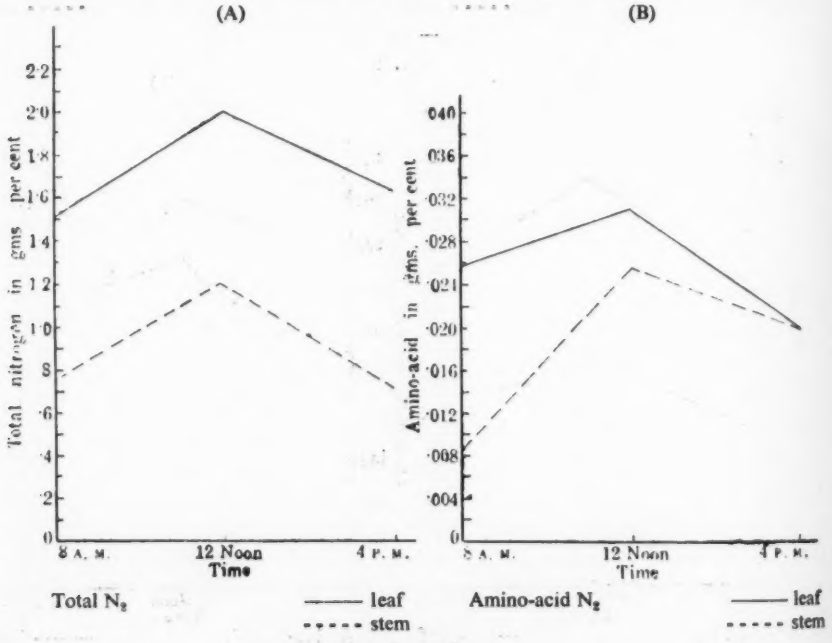
FIG. 5

Figs. 2, 3, 4 and 5 show that even the magnitude of the rise and fall is nearly the same throughout the period the plant is growing. In the case of the amino-acid content also its rise and fall, during the ontogenic drift of the plant, is of the same type as in Fig. 1.

The above results, to a certain extent, support the views of Chibnall and Maskell and Mason who get an increase of total nitrogen in the day and a decrease at night.

Some experiments were also done on plants which were 3 months and over in age and in which flower buds had commenced to be formed. In these cases the swollen nodes near the bases of stems showed that the formation of these buds had taken place.

The total nitrogen and the amino-acid nitrogen of the stems and leaves of 3- and 4-months old plants are given in Figs. 6 and 7 respectively. From these figures it is apparent that the type of the increase of the total nitrogen is the same as that in the seedling stage except that the initial total nitrogen content and the subsequent rise at noon is slightly greater than the total nitrogen in the seedlings. The amino-acid nitrogen, however, in Figs. 6 and 7 shows lower values, inasmuch as in Fig. 5 when the plants were



Age—3 months

FIG. 6

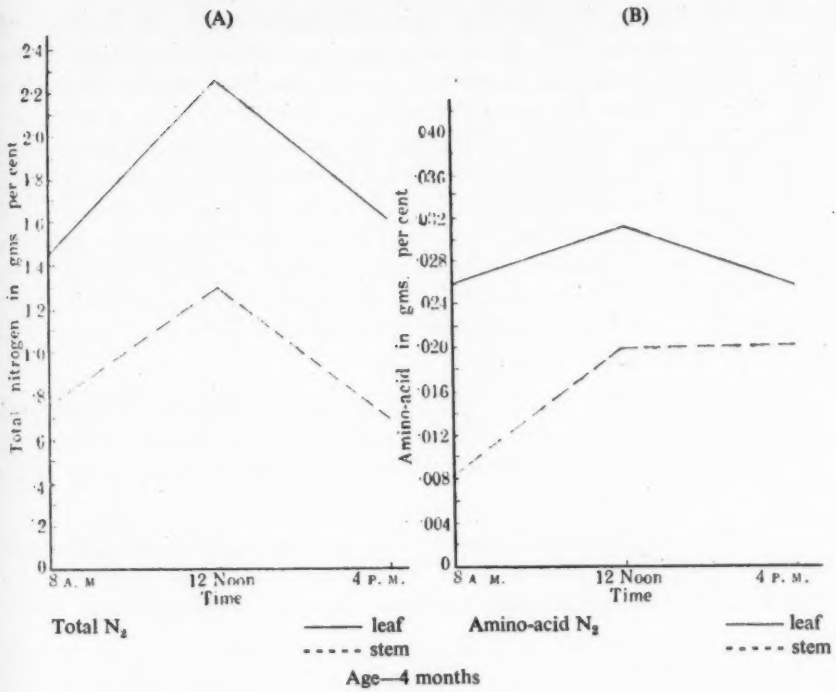


FIG. 7

about 2 months old the amino-acid nitrogen of the leaf started at 0 ·031 gms. per cent. and increased to 0 ·036 at noon, but when the leaves were 3 months old it started at 0 ·026 only, and increased to 0 ·031. This amount one finds in Fig. 7 also where the plants were 4 months old. The slight increase in total nitrogen and a decrease of the amino-acid nitrogen in the leaves of plants 3 to 4 months old, over the young plants, is significant. In trying to explain this difference we venture to suggest that the protein metabolism in plants being obviously a reversible reaction, at a certain equilibrium position the hydrolytic and synthetic rates will become equal. But in plants the problem is far more complicated. Thus, among other things, the quantity of the amino-acid, in the leaf, will depend upon not only (1) the hydrolyses of the proteins to the amino-acids but also to (2) the synthesis of the amino-acids from the raw materials in light. As the production of the acid proceeds from these two sources, the equilibrium position, specific to the plant, is approached. With the approach of the equilibrium position, the condensation rate increases on the one hand, and on the other, the synthetic rate decreases. The decrease in the rate of the latter process is due to the increase of the products

of the reaction, in this case they being the amino-acids. If, however, the amino-acids are removed, say by translocation, then the formation of these acids, by the hydrolysis of the proteins on the one hand, and its synthesis on the other, will proceed at a rate depending upon its removal. In a young fast-developing plant, the amino-acids from the leaves move to the meristematic regions in the stem. Simultaneously with its removal, on the basis of mass action, increasing quantities of this acid will be produced. Conversely, in a mature plant such as plants 3–4 months old where, not only, vegetative growth has ceased but also the spikes have formed, the amino-acids do not travel to the stem. Their concentration in the leaves, therefore, causes increased condensation resulting in the increase of the proteins and a consequent lowering of the amino-acids (Fig. 7).

From the results another consistent feature emerges that from the seedling stage upwards, one always finds a steady increase, in both the stem and leaves, of total nitrogen and amino-acid nitrogen from morning to noon and thereafter a decrease.

These results, therefore, broadly speaking, support the views of Chibnall, Maskell and Mason who also get an increase of total nitrogen in the day, though they do not get a maximum rise at noon as we recorded.

These results confirm the view, that in light, both the amino-acid nitrogen and the total nitrogen increase, but they do not throw any light on the question as to whether this increase is due to the direct photochemical action or whether light only affects the synthesis in an indirect way by increasing the supply of some intermediate substances which may chemically unite with simple nitrogenous substances to increase the organic nitrogen compounds in the leaves. Light could also act in another indirect way and that is to increase the respiratory rate of a green leaf so that an execss of energy is supplied to bring about the endo-energetic reaction in the synthesis of proteins. To further clarify these points the following experiments were performed.

Section II—

Injection experiments on the wheat plant. Glucose injected. Strength 2% *solution.*—Wheat plants were removed in the morning at about 7 A.M. from the field and soon after were placed in beakers containing a little water. After about an hour 2% solution of glucose was injected into them. The plants were then kept covered, for the whole day, with a piece of black cloth.

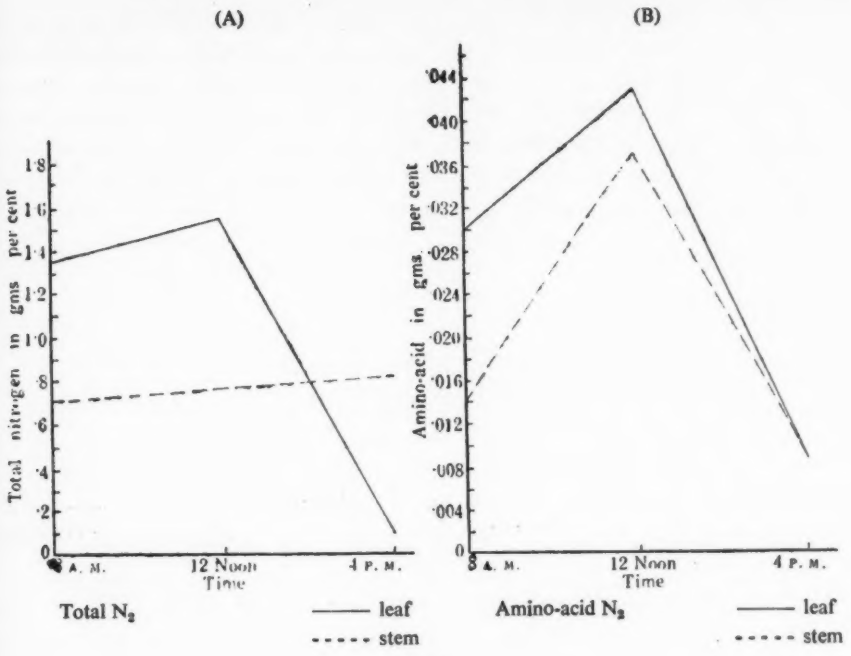


FIG. 8

Observations were recorded as usual at 8 A.M., 12 noon and 4 P.M. The amounts of total nitrogen and amino-acid nitrogen in the stems and leaves are given in Fig. 8.

The total nitrogen of the leaves rises from 1·35 to 1·55 at noon and thereafter falls off rapidly to 0·1 in the evening. The total nitrogen of the stem, however, continues to rise throughout the day.

The amino-acid nitrogen of the leaves arises from 0·03 to 0·043 at noon and then rapidly falls off to 0·008 by the evening. In the stem the amino-acid nitrogen is 0·014 in the morning, then it quickly rises to 0·036 at noon to fall off to 0·008 in the evening.

A control experiment was also carried out with plants similarly kept in dark without the glucose injections. Fig. 9 gives graphically the record of total nitrogen and amino-acid nitrogen of this set.

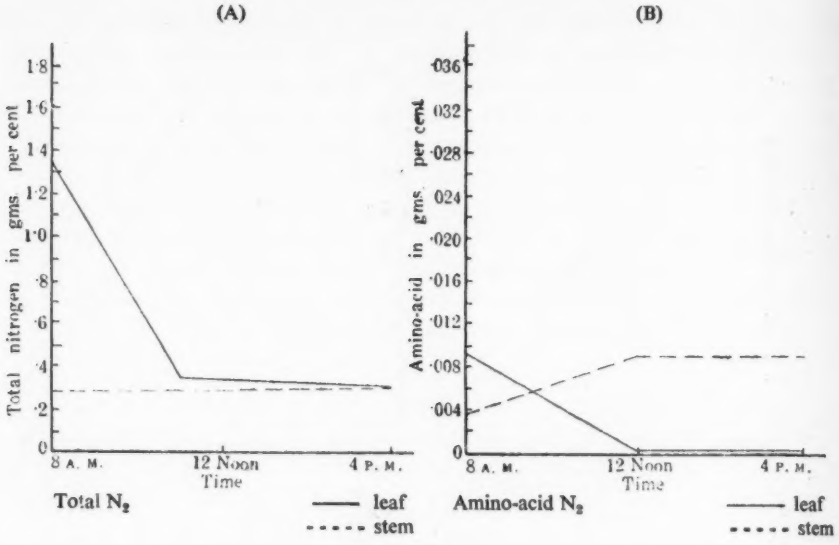


FIG. 9

The total nitrogen of the leaf is 1·35 in the morning and by noon it falls rapidly to 0·33. The rapid fall then slows down and in the evening the value of the total nitrogen is 0·3. The total nitrogen of the stem throughout the day remains practically unchanged at 0·3.

The amino-acid nitrogen of the leaf falls from 0·01 in the morning to 0 by noon, and no amino-acid could be detected even in the evening. The amino-acid of the stem, however, rises from 0·004 in the morning to a little over 0·008 at noon. This amount then remains constant till evening.

Glucose in Shive's solution injected. Strength: 2% *Glucose* + *Shive's solution with a slight excess* $Ca(NO_3)_2$.—As in previous cases plants were brought from the botanical garden in the morning and were then injected with 2% glucose + Shive's solution, containing slightly higher percentage of nitrate. The plants were kept covered for the whole day with a piece of black cloth. The observations were recorded as usual at 8 A.M., 12 noon and 4 P.M.

Fig. 10 which gives graphically the total nitrogen and amino-acid nitrogen in the leaves and stems shows a rise and fall very similar to the rise and fall in Figs. 1–5 but the rise is slightly more accentuated here. Normally when the plants were growing in the open during their (Fig. 3) seedling stage the total nitrogen in the leaf and stem at 8 A.M. was 1·3 and 0·7 respectively while in this case it was 1·45 and 0·7 respectively. At 12 noon, the total nitrogen

increased to 2·4 and 0·95 in the leaves and stems of plants injected with the nutrient solution as opposed to 2·0 and 0·95 in the leaves and stems of plants growing in open sunshine (see Figs. 1–5). Thus the increase in this case at noon is appreciably higher. The fall towards evening is also much less, for the total nitrogen fell off to 1·8 and 0·8 in the leaves and stems while in Figs. 1–5 it fell off to such low values as 1·45 and 0·65.

But, one finds the biggest increase in the case of the amino-acid nitrogen.

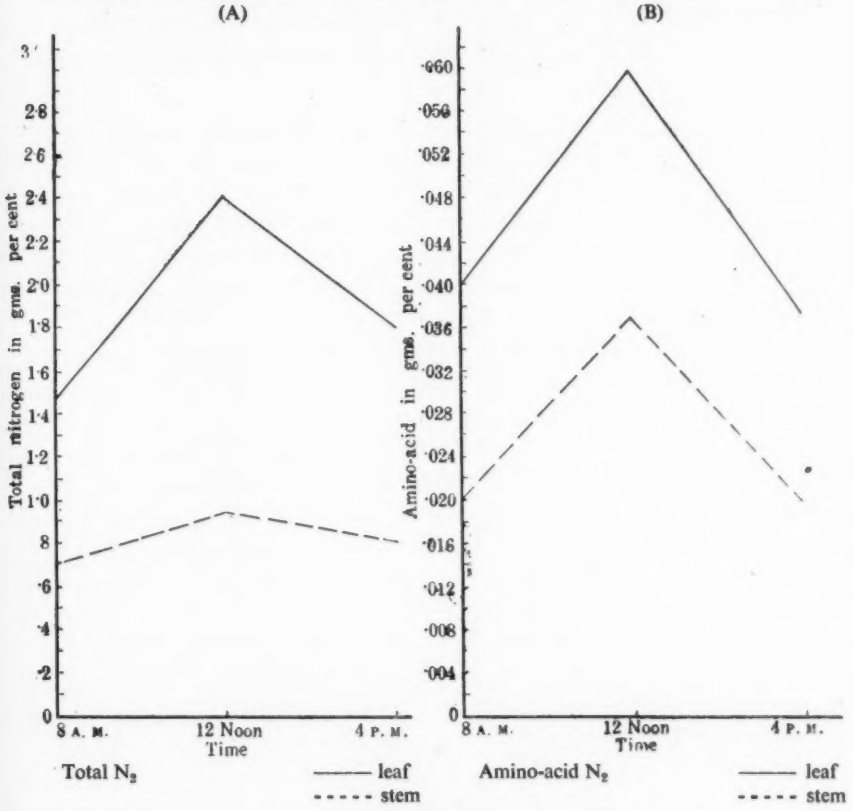


FIG. 10

Here the initial value was 0·040 and 0·020 in the leaves and stems respectively at 8 A.M., which rose rapidly to 0·060 and 0·037 respectively at noon. The corresponding initial amino-acid contents at the seedling stages (Figs. 1–5) was 0·030 and 0·013 repectively which rose at 12 noon to 0·035 and 0·024–these values are about half. One therefore arrives at the definite

conclusion that even in the dark but with the injection of glucose and Shive's solution containing excess of nitrates there is a greater increase of total nitrogen and amino-acid nitrogen than in the case of those plants that are growing in the open sunshine.

Discussion

Total nitrogen and amino-acid nitrogen of the stem and leaf—

At any moment the total nitrogen, *i.e.*, the total inorganic and organic nitrogen contents of a leaf will depend upon the following two variables :—

(*a*) The rate of translocation from the stem into the leaf of nitrogenous compounds and

(*b*) the rate of translocation of nitrogenous substances from out of the leaf to the stem. If the rate (*a*) is more than (*b*) then there will be an accumulation of nitrogenous matter in the leaf and *vice versa*. It is, therfore, obvious, since movements of nitrogenous substances do take place in the stem and leaf, that there will be fluctuations in the amounts of this material at a particular moment in a given leaf.

Schulze and Schutz[13] found for Acer a higher nitrogen content in the evening than in the morning. Chibnall, working on the scarlet runner bean, noted a fall in the total notrogen at night. Miller[7] also working on soybeans, cowpeas and garden beans, noted that in the majority of cases the total nitrogen increases in the evening and decreases is the morning.

The work done in this laboratory shows that at all stages of the development of the plants the leaves and stems show a low nitrogen content in the morning, a rapid rise to a maximum at noon and then a fall towards evening. These results are slightly different from the results of Chibnall, Miller and others who found a greater increase of total nitrogen towards evening.

The increase of protein nitrogen (shown in all the figures as total nitrogen), in the leaves is obvious if there is an increase of amino-acid nitrogen for, when a certain critical concentration of amino-acids has reached in the leaves, condensation to polypeptides and thence to higher proteins will necessarily take place and thus the total nitrogen, which in effect, is the total nitrogen of the proteins, will show an increase. Therefore, with the increase of the amino-acid nitrogen in the day a simultaneous increase of total nitrogen will also be seen in the leaves.

The rapid increase of the amino-acid nitrogen of the leaf in the day may be due to two factors *viz.*, (1) a greater accumulation of the inorganic nitrogenous material by the rapid translocation brought about by increased

transpiration in the day and (2) a rapid synthesis of organic nitrogen from inorganic sources, with the help of solar radiations acting directly or indirectly. The immediate source of energy for this endoenergetic reaction whether it is from the sun or from the chemical oxidations of the carbohydrates w ill be discussed subsequently. The amino-acids thus formed, get, on the on e hand, partially condensed into the higher proteins in the leaves and on the other get translocated in the stem. This is obvious from Figs. 1–5 (B). The amino-acids of the stem as compared to those of the leaves rises till noon in all the cases much more rapidly. This must be due to the rapid translocation of this material from the leaf into the stem even during the time it is being formed in the leaf. The same Figs. 1–5 (B) show that the amino-acids of the leaf falls in the evening far more rapidly than in the stem. In fact in some cases like Fig. 1 (B) the curve for amino-acid of the stem cuts that of the leaf. This shows that the loss of this substance in the leaf is much greater than the stem. So that even though the production of amino-acid decreases in the afternoon, the translocation to the stem goes on at a fairly fast rate causing a depletion in the leaves of not only the amino-acids, but also of the proteins as is evident by the steep fall of the total nitrogen content of the leaf in the evening. On the basis of the balanced reaction a fall in the concentration of the amino-acids will naturally bring about the hydrolysis of the proteins.

From the accumulation of the amino-acids in the stem it is also evident that till noon the flow is greatest and thereafter this slightly slows down. Assuming now that the flow of any substance will depend upon (*a*) resistance and (*b*) a difference in the potential, then if the resistance is a constant factor, the flow will be guided primarily by the difference in the potential. Now, the time of the meristematic activities of the wheat cells is known to be at about noon. This is the time when new protoplasm is being laid and consequently a greater quantity of amino-acids are needed. Consequently the amino-acid nitrogen potential at the growing tip is nearly zero as it is being rapidly used up, or the difference of potential between the leaf and the growing tip is great. There is of course no change in the resistance for the channels, through which nitrogenous material flows, remains unchanged. Therefore till noon there is a greater flow of the amino-acids.

Energy requirements in the synthesis of proteins—

Experiments in section 2 throw some valuable light on the problem of the energy requirements in the synthesis of proteins. Figs. 8 and 9 are significant in this connexion. Fig. 8 (B) shows that when the excised leaves were injected with a 2% solution of glucose but kept in the dark, the

amino-acids increased considerably till noon, as also there was a slight increase of the total nitrogen in the leaf (Fig. 8 A). The leaves not injected with glucose but kept in the dark showed a rapid fall of total nitrogen (Fig. 9 A) by noon as also there was a rapid fall to zero of the amino-acids (Fig. 9 B). This in itself proves that the synthesis of amino-acids can take place in the total absence of solar radiations but with an excess of carbohydrate material. The fate of the glucose after injection and its rôle in the synthesis of proteins can be summarized as follows :—After the injection of glucose solution the intercellular spaces get filled with this solution. Ranjan[10] working on Cherry-laurel leaves in Cambridge has, however, shown that even after injection enough oxygen enters the leaf to ensure perfect aerobic respiration. The solution after injection fairly rapidly enters every cell of the leaf abutting on the intercellular spaces. The glucose molecules, after their entrance, have now three alternative fates; *viz.*, they get

(1) Condensed into the polysaccharides.

(2) Oxidised to $CO_2 + H_2O$.

(3) Chemically united at some intermediate stage with other chemical substances present in the cell.

With the 1st we are not concerned.

The 2nd and 3rd cases are important so far as it affects protein synthesis.

Taking the 3rd case we suggest that during some stage in the glycolysis of the carbohydrates the substance resulting therefrom unites with some derivative of the nitrates to form the amino-acids. Possibly there are at least two endoenergetic reactions involved in this requiring energy; *viz.*, (1) the reduction of NO_3 to NO_2 during its change to some ammonium derivatives and (2) the synthesis of amino-acid compounds from the chemical union of the above with the glycolysed substance of the carbohydrate. The energy evidently is supplied by the oxidations of the carbohydrates. In uninjected cases, the leaves are also oxidising the carbohydrates in the dark, but evidently the energy is only sufficient to carry on the other metabolic activities of the plant cell and hence an excess is not available for the synthesis of amino-acids. But when glucose is injected, as shown by Ranjan,[10] for Cherry-laurel leaves, the respiration rate temporarily increases to a great extent. Thus quite a lot of energy is released which is utilised in the synthesis of proteins. This increased energy is lacking in the leaves in dark, and hence there is no formation of amino-acids.

Again numerous injection experiments have shown that after injection the respiration rate only temporarily increases, the duration of the increase

depending upon the concentration of the solute so injected. Thus, after a time, when the respiration rate climbs down to the normal the synthesis of the amino-acids also decreases as is shown in Fig. 10 where towards the evening both the total nitrogen and the amino-acid nitrogen decreased considerably.

In the case of plants kept in dark but not injected one finds (Fig. 9 B) that the amino-acid nitrogen of the stem increases at noon, though the total nitrogen remained constant throughout. The total nitrogen and the amino-acid nitrogen of the leaf, however, came down very low at noon. As has been peviously said the meristematic activity is maximum at noon in the wheat plant. And thus proteins in the form of amino-acids travel from the leaf into the stems at noon, causing an increase of these substances in the stem at the cost of the leaves.

These experiments also show that after injection the energy of oxidation of glucose is in excess to the requirements of energy during synthesis of proteins. In other words, the energy is not the limiting factor, but the nitrate is the limiting factor as Fig. 10 shows. When 2% glucose solution along with Shive's solution with an excess of $Ca(NO_3)_2$ was injected, the amino-acid content of the leaf started at 0·040 and reached 0·060 at noon whereas, in only the glucose injected leaves, the amount was 0·008 in the morning rising to 0·02 at noon. Thus the increase was nearly 3 times in the case of Shive's solution with glucose. Here the percentage of glucose was the same as in the experiment represented by Fig. 8, and so the energy of oxidation was kept constant but only the nitrate was increased resulting in the increase of the synthesis of amino-acids.

The synthesis of proteins in light—

In the light of the aforesaid discussion we venture to suggest that when the wheat plants are normally growing, in light, the energy for the synthesis of amino-acid is derived from the oxidation of the carbohydrates in light and not by the energy of the solar radiations that are absorbed by the green leaf.

For if the solar radiations were responsible for the synthesis of proteins, then at 4 P.M., as at noon, there should have been an increase in the total nitrogen. But, as is evident from the various figures, it is not so. The question then arises as to the cause of this fall, in the afternoon, in the total nitrogen.

As has been said before, the translocation of the amino-acids cannot be responsible for this fall for the simple reason that relatively little of this acid is translocated in the afternoon. Yap[14] has noted, however, that the rate of

photosynthesis of the sugarcane decreased from 10 A.M. to 4 P.M., the process being the most active from 8 A.M. to 10 A.M. This has also been noted for other plants by various other workers. When carbon assimilation is actively going on, the respirable sugar, on the one hand, increases which causes increased respiration and on the other, substances like propionic acid may be formed as shown in Fig. 11 which may chemically unite with NH_3 to form the amino-acids. If this be so, it will account for the increased formation of the amino-acids and the total nitrogen at noon and their relative decrease in the afternoon. Ranjan[11] has also shown that the rate of respiration, in light, of a normal green leaf increases considerably over the rate of respiration in darkness. Thus the excess of energy so liberated gets transferred for the synthesis of the amino-acids. A tentative scheme of reactions is given in Fig. 11.

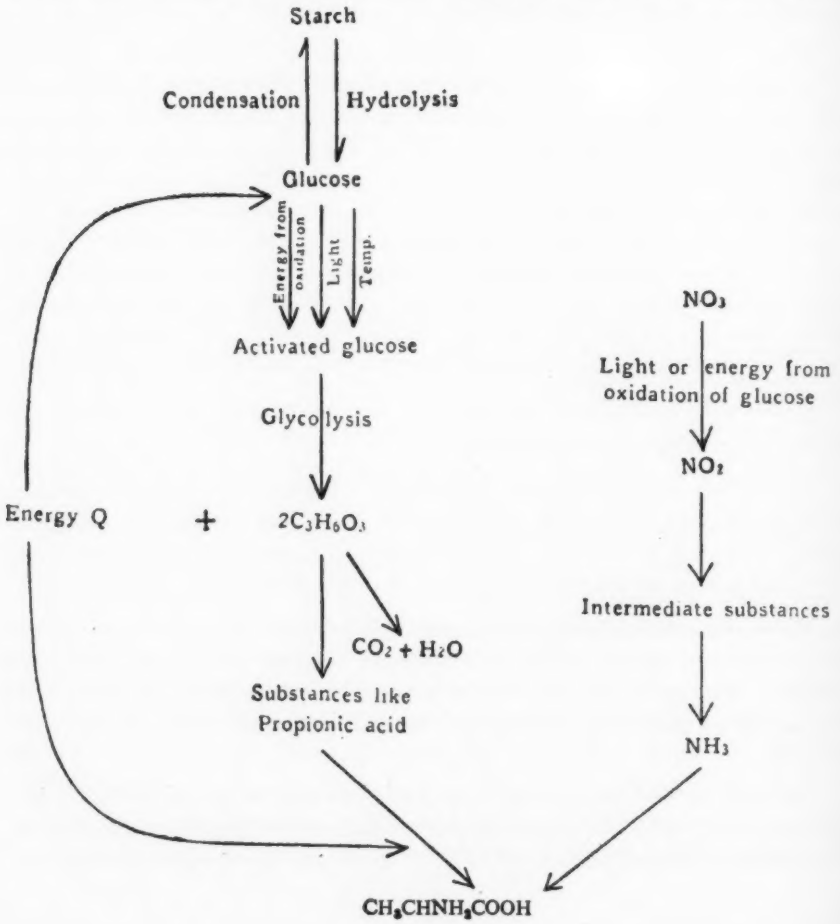


FIG. 11

We may then venture to sugest that the twin causes responsible for the increase of organic nitrogen in light are (1) increased respiration in light and (2) formation of intermediate substances, as a result of carbon assimilation, which eventually form the amino-acids. Thus light plays only an indirect part in the synthesis of proteins.

Summary

1. Samples of stems and leaves were separately obtained from the wheat plants at 8 A.M., 12 noon and 4 P.M. The total nitrogen and amino-acid nitrogen of the above samples were estimated. It was observed that the amounts of total nitrogen and amino-acid nitrogen in the leaves rise and reach a maximum value by noon, and thereafter decrease. In stems the total nitrogen varies directly with the variations in the total nitrogen of the leaves.

2. Excised leaves when kept away from light showed a rapid fall in the total nitrogen content from morning to noon, while the nitrogen in the stem remained constant throughout.

3. When the leaves were injected with 2% glucose solution and kept in dark the total nitrogen increased towards noon and thereafter it fell off. The nitrogen of the stem, however, showed throughout a slight rise.

4. The total nitrogen in the leaves when injected with 2% glucose + Shive's solution and kept in dark, showed a marked increase towards noon. The increase, however, fell off towards evening.

5. In discussing the results the authors find that in the day the carbohydrates increase, as a result of photosynthesis, so also the total nitrogen. But how is it that there is such a relationship ? The senior author has shown elsewhere that the respiration increases in light to a marked extent. The increased energy thus released by increased respiration in light brings about the synthesis of proteins. This is substantiated by experiments in plants kept in dark but injected with glucose + Shive's solution. In this case both the total nitrogen and the amino-acid nitrogen rapidly increases. Here again, with the injection of glucose the respiration rate rises rapidly, and the increased energy given out in this exothermal reaction is enough to cause the synthesis of nitrogen from some derivative of the NO_3 of the Shive's solution and the glycolysed product of the injected glucose. In the case of leaves in light, however, due to carbon assimilation, intermediate substances which ultimately unite with the derivatives of nitrates are formed and with the help of the energy of the respiration in light, organic nitrogen is built up. Light, therefore, plays only an indirect part in such a reaction, the direct being played by glucose. A tentative scheme of reactions is given in the text.

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CONTRIBUTION TO THE MORPHOLOGY AND CYTOLOGY OF *ALPINIA CALCARATA* ROSC., WITH SPECIAL REFERENCE TO THE THEORY OF ZINGIBEROUS FLOWERING

By Dr. T. S. Raghavan, M.A., Ph.D. (Lond.), F.L.S.
(*Head of the Department of Botany, Annamalai University*)
AND
K. R. Venkatasubban, M.Sc.
(*Annamalai University*)

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1. *Introduction*

The most exhaustive work of recent times on the Scitamineæ is that of Mauritzon (1936). It deals with the structure of the ovule, in some cases its development, and the embryology of representative genera of the four families comprising the Scitamineæ. In the course of this work, a brief reference is also made to the genus *Alpinia* under the Zingiberaceæ. Comparatively little is known of this family both as regards the chromosome numbers of the important genera and developmental morphology. Gregory (1936) recorded the chromosome numbers of *Elettaria cardamomum* ($2n = 48$), *Costus malarotiensis* ($2n = 18$), *Hedychium Elwesi* ($2n = 66$). He also worked out the floral anatomy in some detail especially for the elucidation of the morphology of the labellum in *Elettaria cardamomum*. While this paper was being prepared for the press, we have seen a paper on the life-history of *Costus speciosus* by Banerjee (1940) in which a short account is

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given of the work on the Zingiberaceæ done so far. Humphrey (1896) was one of the earliest to pay attention to the Scitamineæ. He described the development of the seed in a number of genera and incidentally described a few disconnected details regarding the organization of the embryo-sac. Some of these details have been controversial and a few have had to be revised; for instance, in the genus *Costus*, he had suggested '*Adoxa*-type' of embryo-sac development. This however was found to be incorrect by Banerjee and Venkateswarlu (1935). They found a normal type of embryo-sac formation.

From no point of view is the Zingiberaceæ more interesting than from that of the morphology of the flower. One of the most recent workers in this field is Thompson (1933). He has subjected to a critical ontogenetic study a large number of representative genera and has come to conclusions, a brief mention of which will be made further down in this paper.

In the present paper the somatic chromosome number of *Alpinia calcarata* Rosc. has been recorded for the first time. Some cytological details as well as details of the embryo-sac development are also given. Effort has also been made to study the ontogeny of the flower and of the floral group in order to throw some light upon the morphology of the much-discussed labellum.

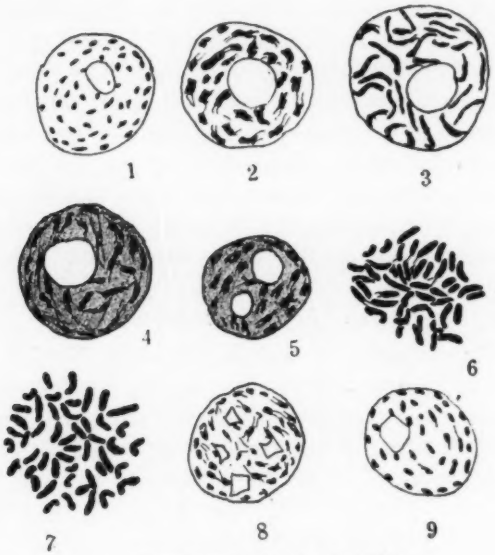
Alpinia calcarata is extensively grown in gardens for its showy flowers and aromatic leaves. It is a perennial herb which flowers in these parts in the months of February and March. No fruits are formed; and the reason for this will be found in the body of the paper.

The technique adopted does not call for any special mention. For the cytological work, root tips were fixed in Navashin's fluid after pre-fixation in Carnoy. Chloroform was used as the paraffin solvent. Newton's Iodine Genation violet was used for staining. Ovaries and young flower buds were fixed in F. A. A. and stained in Delafield's and Haidenhain's Iron-alum hæmatoxylin.

II. The Ovule and the Embryo-sac

The ovary is tri-carpellary with axile placentation. The ovlue at a very early stage develops the inner integument, and almost simultaneously with this, a sub-epidermal archesporial cell is differentiated (Text-Fig. 10). This cell cuts off a primary parietal cell (Text-Fig. 11), which undergoes usually an anticlinal division (Pl. XVII, Fig. 8). Usually it does not undergo any periclinal division, so that there is only one layer of wall cells between the nucellar epidermis and the embryo-sac. In *Costus* Mauritzon (1936) found that the primary parietal cell did not undergo any further division

and it became radially stretched capping the embryo-sac (*cf.* Fig. 11 of Mauritzon).



TEXT-FIGS. 1–9. (× Ca 5400)

FIG. 1. 48 prochromosomes are seen distributed peripherally ; note 4 prochromosomes attached to the nucleolus. FIGS. 2–5. Prophase stages ; note the presence of the threads forming a sort of a reticulum. FIG. 6. Prometaphase. Note that the distal ends of a few chromosomes thread-like structures emanate. FIG. 7. Somatic Metaphase, $2n = 48$. FIGS. 8–9. Early and late telophases.

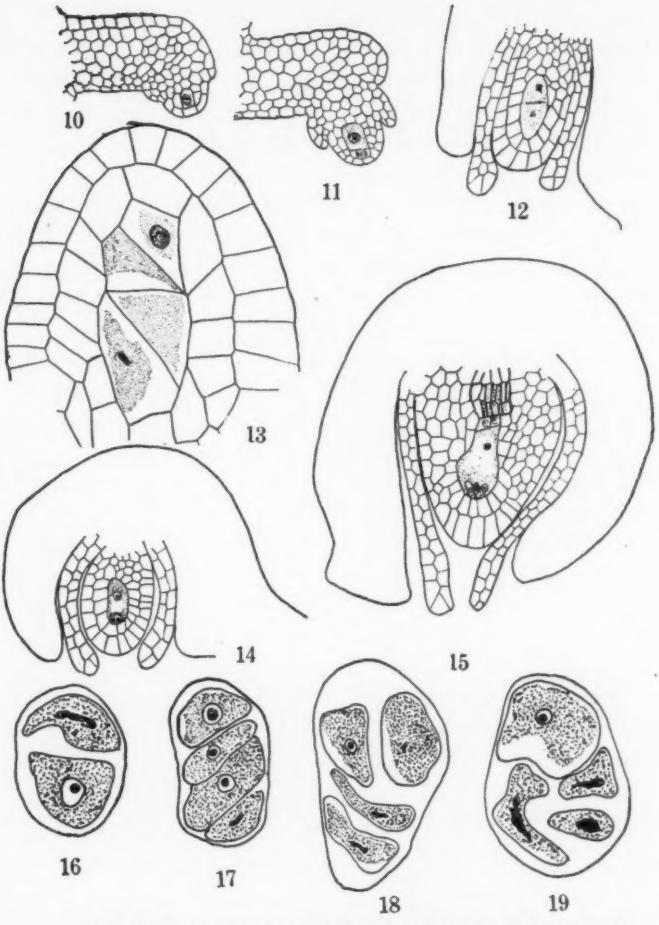
The inner integument is the first to be initiated and its development follows the usual lines. It remains two-layered throughout. It protrudes beyond the outer integument forming a beak-like structure (Text-Fig. 15) and frequently shows a tendency to fuse. Humphrey (1896) in describing the post-fertilization ovule of *Costus* says that " Just after fertilization the fore-end of the outer integument and the free portion of the funiculus begin to fuse and close the micropylar opening finally obliterating the inner integument ". This mass formed by the coalescence, he termed the ' arillode '. Though in this species no seed is set and as such post-fertilization stages could not be followed, the early tendency of the inner integumental halves to fuse is suggestive of the general tendency of the family towards the formation of that large and fleshy aril which caps the micropylar end of each seed.

The megaspore-mother cell undergoes the usual divisions to form the linear tetrad (Figs. 11–13). Even at this stage degenerations in the

gametophyte begin. Text-Fig. 13 shows a linear tetrad that is undergoing degeneration. Text-Fig. 12 shows a dyad in metaphase. In some cases the chalazal megaspore enlarges and develops into an embryo-sac. There can thus be no question of the formation of the '*Adoxa*-type' of embryo-sac, so far as this genus is concerned. The further development is normal but extensive degeneration is met with. Fig. 14 shows a binucleate embryo-sac. The mature embryo-sac is not oval in configuration, but has a narrow antipodal region suggestive of a haustorium (Pl. XVII, Fig. 10). Colour is lent to this by the occurrence of thick-walled cells radially elongated, just around this haustorial structure. Text-Fig. 15 shows a mature embryo-sac. No further development of the embryo-sac takes place beyond the 8-nucleate stage.

III. Pollen Formation

Normally only one anther in a flower develops, but in a few cases two fertile stamens were found. Earlier stages in the micro-sporangial development are of the normal type and hence are not described in any detail. There are about 6–8 layers of parietal cells of which the innermost one is the tapetum. The tapetum is of the intrusive type such as has been described for *Elatteria* (Gregory, 1936) (Pl. XVII, Fig. 11). The tapetal cells are bi-nucleate and they do not become pluri-nucleate. By the time the tetrads are organized, the tapetal cells get completely disorganized and only traces of them could be made out. The formation of the pollen tetrads calls for some remarks. In most monocotyledons, the tetrads are arranged iso-bilaterally. Banerjee (1940) has figured such an arrangement for *Costus*. In *Alpinia calcarata*, however, such an arrangement is met with almost as an exception. Text-Fig. 16 shows a pollen dyad in which wall formation has taken place after the first division as is the case in most monocotyledons. Text-Fig. 19 shows a pollen tetrad arranged on an iso-bilateral plan. But very often the pollen tetrads are arranged in a linear fashion (Text-Fig. 17), resembling the linear tetrads of the megaspores. Frequently T-shaped configuration is also met with (Text-Fig. 18). Similar arrangements—linear tetrad, and T-shaped tetrads have also been met with in *Alœ sisilana* (Vignoli, 1937). Most of these microspore tetrads exhibit irregular outlines with their nuclei very much elongated and in a state of degeneration (Text-Fig. 19). Though normal pollen grains are formed in several cases, it is our impression that in a good many anthers, the tetrads in each locule undergo degeneration *en masse*. The non-seed-setting of the species is, therefore, to be explained both by the failure of the development of the embryo-sac, and also by pollen degenerations.



TEXT-FIGS. 10–19. (× Ca 380, except Fig. 13 which is × 900)

FIGS. 10–15. Development of the embryo-sac. FIG. 11. Same as Plate XVII, Fig. 8. FIG. 12. Same as Plate XVII, Fig. 9. FIG. 13. Linear tetrad degenerating. FIG. 14. 2-Nucleate embryo-sac. FIG. 15. Mature embryo-sac with attenuated antipodal end. Only two antipodal cells are seen. Note also the presence of thickened cells around this end. FIGS. 16–19. Pollen formation. FIG. 16. Pollen dyad. FIG. 17. Linear tetrad of microspores. FIG. 18. T-shaped tetrad of microspores. FIG. 19. Iso-bilateral arrangement 3 of the microspores degenerating.

IV. Prochromosomes

The diploid chromosome number is 48 (Text-Fig. 7). From an examination of root-tip mitosis, it is found that this species of *Alpinia* exhibits prochromosomes. The question of prochromosomes has been discussed

somewhat thoroughly in a previous paper (Raghavan, 1938). It was shown in that paper that the prochromosome, also called the chromocentre by some authors, represents a part of the chromosome namely, that portion on either side of the spindle fibre attachment. The most important proof of this is the fact of the identity in number between the prochromosomes and that of the chromosomes. Since the prochromosomes are distributed peripherally over the spherical nucleus, the determination of the exact number is rendered more difficult than that of the chromosomes at metaphase, where they form a flat plate-like configuration. All the same about forty-eight bodies could be made out in Fig. 1 showing early prophase. Text-Figs. 1–9 show a number of stages in the mitotic cycle. In Text-Fig. 1 we see about 48 darkly staining oval bodies peripherally distributed around a central rounded nucleolus. Very often, we find four of these bodies attached to the nucleolus. Sometimes, there are two nucleoli, frequently differing in size, to each of which two prochromosomes are attached (Text-Fig. 5). Doutreligne (1933) raised a very important question regarding the structure of the nuclei exhibiting prochromosomes. She held that there was no reticulum such as appears in the nuclei which do not show prochromosomes. This is intimately connected with the occurrence or otherwise of chromonemata in plants with prochromosomes. Though no detailed study such as has been made on *Polanisia* (Raghavan, 1938) has been possible in the present case, the few stages that have been represented will reveal beyond doubt that a reticulum is present and that the chromosome cycle at least so far as somatic division is concerned, is essentially similar to that of plants with large chromosomes. In other words, the chromosome structure (chromonematic) as well as the chromosome cycle in plants with prochromosomes are fundamentally the same as in plants without them.

The commencement of prophase is indicated by these bodies becoming more chromatic and slightly bigger and since in this plant almost all the chromosomes are characterised by a terminal centromere, no prominent traces exist of attachment constrictions in these bodies (Text-Fig. 2). Here and there however there are a few sub-median constrictions. At a later stage, these bodies become somewhat irregular and angular and they are now more chromatic than before, and are seen to be connected by a delicate thread-system (Text-Fig. 4). The angularities are soon lost and in well differentiated preparations, it could be seen that threads emerge from the distal ends of these angular bodies (Text-Fig. 5). It can then be seen that this structure results from the intertwining of two chromonemata with the spindle fibre attachment at the proximal end. In fact, it is something like a highly condensed prophase chromosome. No satellites were visible. As prophase

advances, there is a stretching of these bodies (Text-Fig. 3). Of the chromonematic make-up of these bodies, there can now be no doubt. With the passage into prometaphase, there is a further increase in the chromaticity and thin strands are occasionally present connecting one chromosome with another (Text-Fig. 6). Metaphase is characterised by still further contraction (Text-Fig. 7). Text-Fig. 8 shows telophase when the organisation of four irregular nucleoli can be recognised. These bodies have by now attained the angular shapes and fine strands are seen emanating from their ends to form a sort of a network. Very often, these nucleoli fuse together forming an irregular mass. As telophase advances, these strands disappear (Text-Fig. 9). The chromaticity of the prochromosomes is also diminished. Their angularities are lost and with the assumption of a peripheral disposition of these bodies, the passage from late telophase into the resting condition may be regarded as complete. It would thus appear that starting from anaphase, where we find the chromosomes of normal length, they assume a shortened appearance as telophase advances. This is correlated with an apparent increase in the amount of chromatin in the regions immediately adjacent to the spindle fibre constrictions. This has taken place at the expense of the other regions of the chromosomes, which consequently have almost lost their chromaticity. But fibres extending from the ends of these shortened chromosomes are visible. This portion that persists through the telophase is the prochromosome.

V. The Inflorescence

There is some amount of ambiguity in the usual description of the inflorescence of the Zingiberaceæ. An unbranched inflorescence with twin flowers in the axil of each bract, Payer (1857) described as a succession of unipaired scorpiod cymes on a common rachis. Eichler (1875) also maintained that the inflorescence was not so simple but was complicated by fertility of the floral bract. By such fertility between a flower and its bract a cymose system is initiated with sub-floral branching. Thus a cymose system is countenanced in the axil of a spathe. The view that is most commonly held is that of Weisse (1932) according to whom the several flowers in the axil of a single bract, were members of a cincinnate development. Elaborating this Rendle (1904) says that " branching may occur and a monochasial cyme (a cincinnus) is developed in each bract axil ".

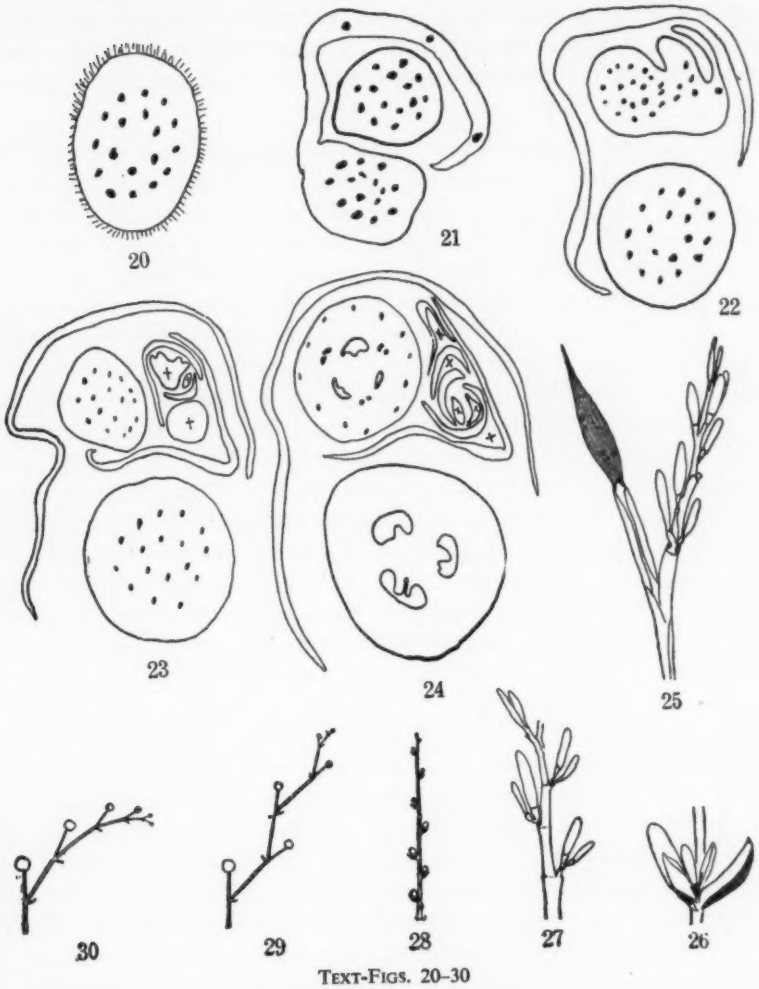
Thompson (1933) after an elaborate ontogenetic study of several genera found no indication of sub-floral branching such as cymosity demands, and considered it racemose. The suggestion of cymose branching characteristic of maturity is, according to him, entirely due to later tortuous growth of the support. Thus he thinks, several species of *Alpinia* are characterised by

monopodially branched inflorescence. The lateral branches are commonly monopodial systems and each major axis of the inflorescence is arrested immediately following the formation of a twin-flowered cone.

In this species on the floral axis are groups of two flowers at different intervals in conformity with the distichous phyllotaxy (Text-Fig. 27). Text-Fig. 25 is that of a floral axis younger in age. This axis is monopodial in growth. Though only two flowers mature at each node, of which the upper is always at a more advanced state of development than the lower, an ontogenetic study of the floral group shows, that usually four to seven floral primordia are initiated. Text-Figs. 20–24 are serial transverse sections of inflorescence axis taken at successively higher levels. Each cluster of flowers is enveloped by a bract and successive sections show that each flower or floral primordium is subtended by a bract. This is accomplished in the following manner. The oldest flower is subtended by a bract which embraces not only the flower in question but envelopes the entire floral group (Text-Figs. 22–24). Similarly the next bract envelops all the flowers of the group except the oldest, which comes to be just outside this bract. In a like manner the bract of flower No. 3 envelops all the flowers except flowers 1 and 2 which consequently come to lie outside this. Text-Fig. 26 is that of a floral group at a node, of which only two mature as shown in Text-Fig. 27. Each group unopened, appears single as in Text-Fig. 25.

Of the 4 to 7 floral primordia initiated the basal one is the earliest to be formed and develops into a flower. The whole floral cluster at each nodal portion presents a zig-zag appearance. In a normal monochasial cyme of the scorpiod type, suppression of the lateral flowers is effected on alternate planes (Text-Fig. 29), so that on a straightened axis, an appearance is presented of a spikate inflorescence with the flowers arranged alternately along the axis. This axis, however, is not to be regarded as the peduncle such as is found in the case of a real spike, but is composed of successive pedicels of the individual flowers. In a monochasial cyme of the helicoid type (Text-Fig. 30) such a straightening would result in the flowers occurring more or less along one plane of the axis. The axis in the previous case is compound being a sympodium.

If we imagine both these types of monochasial cyme undergoing an extreme form of compression, then the flowers which were distributed along an elongated axis become flattened out; and the position of the flowers is dependent upon whether it is a helicoid or a scorpiod cyme. If it is of the former type, all the flowers of a group will be arranged in a circular manner beginning with the oldest at the base. If a straightened cyme of the scorpiod



TEXT-FIGS. 20–30

Alpinia calcarata

FIGS. 20–24. Serial T.S. of the inflorescence axis. The × marking in the Figs. 23 and 24 represent the primordia of flowers which abort. × Ca 50. Fig. 25. Inflorescence at an early stage. FIG. 26. One of the floral groups spread out. FIG. 27. Inflorescence axis at a slightly later stage with only two flowers at each node. FIGS. 28–30. Diagrammatic represenatation respectively of spike, scorpioid cyme and helicoid cyme.

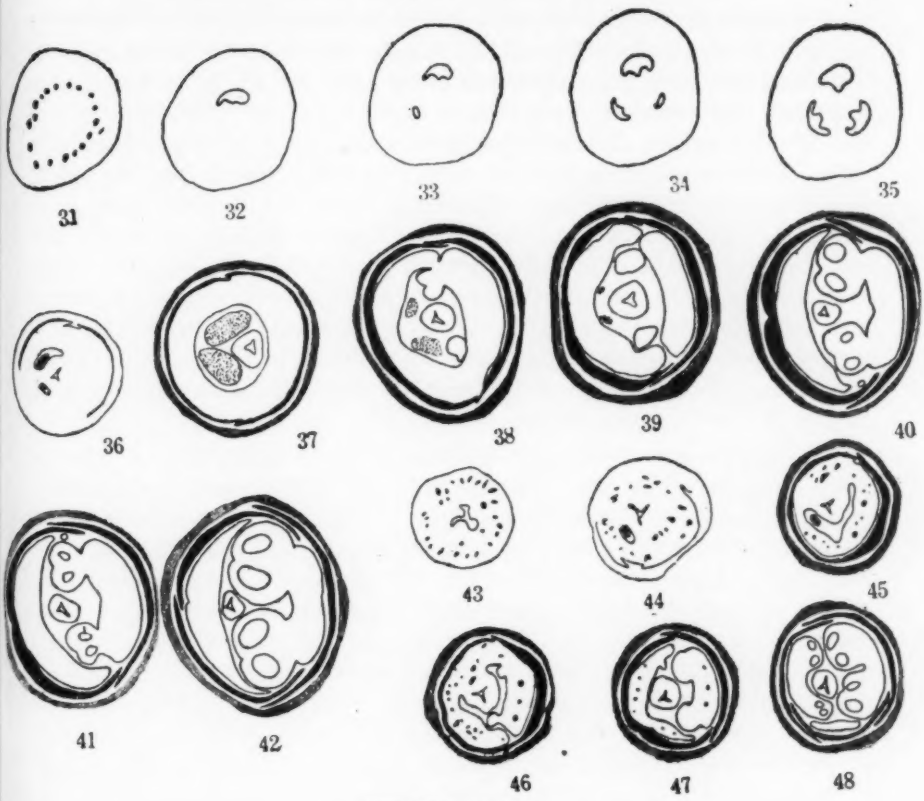
cyme undergoes this compression, then we find that the flowers in the flattened inflorescence are arranged in a zig-zag manner. It is such a zig-zag arrangement that we find in regard to the flowers of each floral group in this species

of *Alpinia* and there can be no doubt as to their cincinnate arrangement. Support to this is also be gained by the disposition of the bracts which in conformity with the cincinnate arrangement are opposite to one another at successive higher levels.

The question whether it could be a compressed type of racemose inflorescence, such as is contemplated by Thompson (1933) may now be considered. The nearest approach is the spike (Text-Fig. 28) not only because of its apparent resemblance but also of the prevalence of this spikate inflorescence in related families of the Scitamineæ. If an elongated spike, the individual flowers of which are subtended each by a bract, undergoes such a compression as we have assumed in the previous cases, a flat inflorescence resembling the capitulum must be the result. The younger flowers will be in the centre, the successively older flowers towards the periphery. Such an arrangement is not to be found in the present case. Moreover in such a case, the bracts, in keeping with the general arrangement of the flower, will also be circularly disposed. Such however is not the case. The zig-zag arrangement of the flowers with the oldest at the base, instead of the circular arrangement, and the opposite disposition of the bracts indicate that each flower cluster is in reality a scorpioid cyme, highly condensed and flattened out. Of the 5 or 6 flowers in each group, only two usually develop, the rest aborting. That is why in a slightly older condition, we find twin flowers occurring at each nodal region of the inflorescence axis.

VI. Floral Ontogeny

Serial transverse sections were taken of the flower buds at different stages of development, in order to have an idea of the method of origin and differentiation of the parts composing the flower. Text-Figs. 31–42 show an ascending series of transverse sections, *i.e.*, transverse sections taken serially at successively higher levels, commencing from the top of the ovary. Text-Figs. 31–42 are those of a normal flower, while Text-Figs. 43–48 represent those of a flower with two fertile stamens, instead of the usual one. Of this latter type, there were cases more or less of frequent occurrence, and not stray and rare. Text-Fig. 31 is the stalk of the flower showing a number of vascular strands distributed peripherally. Text-Figs. 32–35 represent the ovarian cavities appearing not simultaneously, but in a spiral sequence. Their disappearance also follows the same procedure. This spiral arrangement of the carpels has already been interpreted to be of significance in the matter of the foliar nature of the carpels (Raghavan, 1939). As in the case of *Cratæva* reported in a previous paper (Raghavan and Venkatasubban, 1940) the other floral " whorls " also exhibit a spiral sequence in their origin



TEXT-FIGS. 31–48

FIGS. 31–42. Serial T.S. of a normal flower. Note the appearance of the ovarian cavities spirally in Figs. 32–35. The shaded bodies of Figs. 36–39 are the 'glands' which appear and disappear. × Ca 50. FIG. 37. Same as Plate XVII, Fig. 1. FIG. 38. Same as Plate XVII, Fig. 2. FIGS. 43–48. T.S. of two-stamened flower. Note the presence of only one 'gland' (shaded structure). × Ca 35.

and differentiation. And the carpels composing the gynæcium, only conform to this general rule, adhered to by the other floral members. Text-Fig. 36 shows the top of the ovary and from now upward, the differentiation of the other floral members is to be seen. Sepals are differentiated constituting a single tubular structure, surrounding or enclosing all the other floral parts except the ovary, which being inferior is below it. The posterior petal is the outermost (Text-Fig. 37) and no deviation from this was ever seen. The labellum (Text-Figs. 38–39) next gradually differentiates itself. It is especially with regard to this that the flower-buds of different stages of development

were examined. Even the earliest possible stage did not reveal the superposed dual or double nature of this organ. No staminodes were present. The two lateral rounded structures in Text-Fig. 41 and Pl. XVII, Fig. 3, may at first be interpreted as staminodes, but these are only the inrolled marginal tips of the labellum. The posterior fertile stamen likewise makes its appearance. About the same time two structures are differentiated, also in a spiral order in an antero-lateral plane (Text-Figs. 36–39 and Pl. XVII, Figs. 1–2). Particular care was taken to ensure that these, unlike the staminode-like structures referred to previously, were not portions of other organs. These whose nature will be discussed elsewhere, are tentatively called the glands. They are quite independent of the other floral organs. Their appearance and disappearance as seen in serial sections are shown in Text-Figs. 36–40. These two antero-lateral glands together with the posterior fertile stamen would appear to form a natural whorl.

The plan of construction of the flower with two fertile stamens is essentially the same. The other floral whorls are differentiated in the usual manner. But along with the usual fertile stamen formed posteriorly, there is another differentiated in a position which may be described as antero-lateral (Text-Figs. 43–48 and Pl. XVII, Figs. 4–7). This anther however is not so well formed as the usual posterior one. By its side, there appears and disappears a small structure. Two things are worthy of note: Firstly that in this type of flower, the two prominent glands usually present as a rule in the normal flower, are conspicuous by their absence; but instead only one structure, smaller than the usual gland, appears alongside of the second fertile stamen. Secondly, the labellum in this type of flower (Pl. Fig. 7 and Text-Fig. 48) is much smaller than in the normal flower. The interpretation and the significance thereof will be discussed elsewhere.

VII. The Labellum : A Discussion of its Morphology

The morphology of the labellum in the Zingiberaceæ has been the subject of much discussion. The generally accepted view is that of Payer (1857) and Van Tiegham (1868). This is based on the trimerous plan of floral construction. They regard the labellum as a part of the inner staminal whorl, forming the two antero-laterals. The posterior one of the same whorl forms the single fertile stamen. The outer staminal whorl is composed of the postero-lateral staminodes while the anterior member of this inner staminal whorl is suppressed. The other view is that of Brown (1830). It regards the labellum as a member of the outer staminal whorl with the two staminodes. The inner whorl is said to be made up of the fertile posterior stamen and the two antero-lateral glands which are present very commonly among the Zingiberaceæ.

The first view supposes the labellum to be really dual in nature, so that the inner whorl is made up of the three members, *i.e.*, the labellum (2 members) and the posterior fertile stamen. It may also be noted that the glands upon which Brown's theory is built is ignored altogether. If the dual nature of the labellum is conceded, then a serious difficulty will beset Brown's concept and that is, the inner whorl will then consist of four members—the two staminodes and the labellum (2 Nos.) and this will seriously affect the trimerous plan of floral construction. Moreover the plan of alternating position of successive floral whorls would require the members of the outer staminal whorl to be opposite to those of the sepal whorl and not to those of the petals as it would be the case, if we were to accept the double nature of the labellum. This difficulty was solved by Brown by considering the labellum as a unitary structure and not composed of two members of the inner whorl.

In addition to these two views, there has been put forward recently yet another theory (Gregory, 1936) which in essence may be regarded as a modification of Payer's concept. This view regards the labellum neither as a single nor as a dual structure, but as an organ composed of three members, the structure corresponding to the midrib portion being considered as the third member. This together with the two staminodes forms the outer staminal whorl, the fertile stamen and the antero-lateral members of the labellum forming the inner whorl. The main virtue of this theory is that all the members of the two staminal whorls are accounted for and there is no need to regard any organs as being suppressed. The presence of the glands is noticed, but no importance is attached to them.

The edifice upon which this concept is based is mainly anatomical. At the region, according to the author, where the bundles of all the members are seen, there are three groups of vascular strands for each floral whorl. The outermost sepal whorl has more bundles, the alternating petal whorl has also a series of nine bundles in three groups. Alternating with these and opposite to the sepal whorl are three groups of (two each) bundles for the two staminodes and the central portion of the labellum, regarded by the author as the third member thereof. Alternating with these is another group of three (also two bundles in each group) supplying the inner staminal whorl; *i.e.*, the posterior fertile stamen and the two antero-lateral members of the labellum. It is also said that the origin of the bundles to these two staminal whorls is different. At the pedicel region, there are twelve vascular strands, three forming a central group and nine forming the peripheral bundles, the latter form the sepal strands. The central group divides further contributing to the petals, and to the inner staminal whorl. In a similar

manner the outer staminal whorl is supplied by a continuation of the peripheral sepal groups. In other words, the bundle groups opposite to the sepals (those supplying the inner staminal whorl, made up of the two staminodes and the " midrib " of the labellum) are a continuation of the peripheral groups on the same radii while those opposite the petals, *i.e.*, the inner staminal whorl (the fertile stamen and the antero-lateral members of the labellum), originate from the central group. Thus all the six members of the two staminal whorls are accounted for and the plan of construction of the flower is not in any way affected. Not only do the floral whorls alternate with one another but their respective vascular supplies also exhibit this alternating arrangement so far as their origin is concerned.

In the present study anatomical evidence is not made use of for the elucidation of the morphology of the labellum. Only such ontogenetic evidence as will be useful in the discussion is let in, and the wide prevalence of what may be regarded as abnormal flowers, has been taken advantage of in venturing to offer an interpretation.

The first question is, what evidence have we for regarding the labellum as being composed of two members? Apart from the anatomical evidence that may or may not be accepted, the only other reliable evidence is that of ontogeny. We have examined sections of very young flower buds and we have not found any sign on the basis of ontogeny for the dual nature of the labellum. At the early stage of development there must be some evidence or other to show that it is made up of two members. Such a free double stage may not exist for a long time and the fusion may almost be congenital. But there must be evidence in an ontogenitic study even of this congenital fusion. As in the case of a polycarpellary syncarpous pistil a critical examination of the ontogenetic stages has revealed (Raghavan, 1939) the individual existence of the carpels and the ultimate formation of a syncarpous ovary by the marginal fusion of the carpellary leaves, so also in this case, must be seen at the earlier stages, evidence of the dual nature of the labellum. When this is so, there is very little ground for accepting the view that the labellum is made up of three members. The support for this concept, derived from the venation of the labellum may not be of much avail. This would involve the belief that the middle portion is a separate member, wherein, only the vascular strands have persisted while the other portions have perished. What evidence have we to take it for granted that while the rest of the labellum has ceased to exist, the vascular bundles alone have persisted? It is this same what may be termed the doctrine of the conservatism of vascular bundles that has been responsible for regarding every stout fibro-vascular cord running longitudinally as the midrib of a carpel

and the propounding of the theory of carpel polymorphism. The ' fantastic ' nature of such conception has been well brought about and discussed by Parkin (1926, 1933) and the same arguments may well apply in this case also.

Moreover the anatomical work of Arber (1933) has shown that a ' rudimentary external form was found to correspond to a vascular system which is equally or even more rudimentary; indeed an organ which retains some trace of its normal external form may yet show a complete lack of vascular tissue '. When this is so, there is no justification to hold that the vascular cord alone has survived while the rest of the labellum has gone as Gregory's (1936) concept would have us believe.

Support to this is also found to be in a note by Joshi in the latest number of the *Annals* (*Ann. Bot.*, New Series, Vol. IV, No. 15, p. 669) where he describes the anatomy of some abnormal flowers of *Gagea fascicularis*. These have five perianth leaves, five stamens and two carpels and they are derived from the normal flowers by the loss of one of the inner perianth leaves, one stamen and one carpel. The study of the vascular system of this flower showed the total absence of any rudimentary vascular traces of the lost parts. It is therefore clear then, that no bundles persist after the organ which they supplied has ceased to exist. When this is the case with a flower not separated even by one generation from the normal flowers, there is little ground for supposing the vascular cord alone persisting even though the ' third member of the labellum ' had disappeared many generations ago.

An ontogenetic study of normal as well as the so-called abnormal flowers in this specis of *Alpinia* indicates certain facts on the basis of which the structure of the flower, especially the morphology of the labellum, could be indicated.

The posterior petal is the outermost petal and the fertile stamen is also posterior as it should be. Serial transverse sections of young buds have revealed the unmistakable presence of two structures situated antero-laterally. They are very prominent to start with but are not continued as far upwards as the anther. They soon disappear in transverse sections taken at successively higher levels. They are situated internal to the labellum and together with the fertile stamen would form a natural whorl of three structures. To our mind these are the two glands of Brown (1830) which along with the fertile stamen constitute the inner staminal whorl. The outer whorl is made up by the anterior labellum and the two postero-lateral stami-nodes; the latter however may or may not be present.

A description of the flower of the genus *Zingiber* as given in the ' genera Plantarum ' (Bentham and Hooker, 1880) may now be briefly recalled with

advantage. It says that there is a short cylindrical tubular three-lobed calyx, the corolla tube cylindrical and the lateral staminodes are connate with the labellum or may be wanting. The labellum is considered a single unit, but is bifid or in two parts. Sometimes the staminodes are petaloid. In the present case, Text-Figs. 41 and Pl. XVII, Fig. 3 show two small rounded structures appearing at particular level and connate with the labellum and which at first sight may be taken up for the posterio-lateral staminodes. But a critical examination of the serial sections has shown that these are only the in-rolled ends of the labellum. So that in this case the staminodes are absent. The outer staminal whorl is therefore represented by the labellum and the absent staminodes. Rendle (1904) has however adopted the Eichlerian concept and says that the morphology of the flower has been the subject of much discussion especially as regards the part played by the labellum. The outer whorl of stamens may be suppressed as in *Costus* or *Renealmia*, but is generally represented by two lateral staminodes, the development of which shows a great variety. The inner staminal whorl is complete, the median stamen is fertile while the lateral pair unite to form the labellum.

Let us see now, if a critical study of the so-called abnormal flowers gives us support for the position we have taken as regards the labellum. In these flowers, there are two fertile stamens whose position would confuse us if we saw only the opened flower. Only by a close ontogenetic study is it possible to have a correct idea of their real position and their significance. The two fertile stamens at first sight appear to be side by side. One of these two is the usual one found just below the posterior overlapping petal. The occurrence of an additional stamen adjoining to this may not be easily explained except that it is likely to be a case of a bifurcation of the stamen primordium—something like the 'dedoublément' of Payer (1857). But a careful examination of the serial sections indicates that the additional stamen's real position is in the anterior side slightly towards one side. The two glands which are found in normal flowers are antero-lateral and it is just in the place occupied by one of these that this additional fertile stamen occurs. In other words, one of the two antero-lateral glands has become staminiferous. If this premise is correct, then we should find the other gland in its usual position inasmuch as it has not undergone any trans formation. This is exactly what we find. Text-Figs. 44–46 and Pl. XVII, Figs. 4–6 show the appearance and disappearance of one of these glands, whereas in the place of the other gland we find the additional fertile stamen. This stamen compared to the normal stamen is smaller.

It will also be seen from a comparison of Figs. 3 and 7 of Plate XVII that the labellum in these two-fertile-stamened flowers is much smaller than in

the normal flowers. We find after an exhaustive search for an explanation that it is a consequence of spatial necessity. The space which was practically free, having been occupied by one of the two small glands, has now come to be occupied by a large anther, so that there is no place for the labellum to grow in that lateral direction. So much so, the labellum is confined practically to the space lying between the two anthers (Text-Fig. 48).

The transformation of one of these glands into a stamen supports the conclusion previously arrived at—that the inner staminal whorl is composed of the two glands and the fertile stamen. In other words the so-called glands are morphologically equivalent to stamens. We are not quite happy about the term gland which usually carries with it no morphological significance. But for want of a better term and so as to conform to the old terminology of Brown (1830) the same expression has been retained.

It will be of great benefit and interest to digress a little and discuss at some length the views of Thompson (1933) in so far as they pertain to the Zingiberous flowering. His Figs. 103 A–R are those of a flower of *Zingiber officianale*, showing descending transverse sections. The remarkable feature is that organs (Nos. 18 and 19 of his Figs.) appear and disappear exactly like the glands of this paper. But he calls these the stylodes and they are members of the floral organs from which the accepted style is composed. His Fig. 104 gives an idea of his concept of the floral structure of *Zingiber*. The bract and the three sepals are as usual. The petals come in for a good deal of modification. The original petals are 5, 6 and 8 with 9 and 10 united with 7. That is the overlapping posterior petal of our figures, become a compound petal, made up of three units. The labellum is also a compound structure, being a fusion product of 8, 11 and 13. The stylar components and the stylodes are numbered 14–19. It would appear from this that the flower is composed of (1) a whorl of 3 sepals (2, 3 and 4); (2) two whorls of petals, 5, 6 and 7 and 8, 9 and 10 (7, 9 and 10 forming a compound petal and 8 is the central portion of the labellum); (3) one staminal whorl 11, 12 and 13 (of which 12 is the fertile stamen, 11 and 13 are the lateral components of the labellum); (4) the first three stylar components are 14, 15, 16; the additional stylar primordia are 17, 18 and 19. But 14, 15 and 17 become confluent with 17 as the prominent member of this union. 18 and 19 enlarge, are separated from the stylar dome and are considered to be stylodes. These according to him are the staminodes of Rendle (1904).

So far as this point is concerned, it will be found from a comparison of the figures in the present paper with those of Thompson (1933), his stylodes, 18 and 19 occupy the same position in the flower as do the so-called glands of this paper. They occupy an antero-lateral position. Rendle's staminodes

on the other hand occupy the postero-lateral position. It does not seem therefore quite appropriate to equate the so-called stylodes occupying an antero-lateral position with the staminodes which are postero-lateral in their placement. It might have been more appropriate to say that the so-called glands of Brown are in reality the stylodes. But so far as our observations go, we are not in a position to conceive of these as the stylar components. Nor is there any evidence in the present material to consider the posterior petal as compound, being made up of three components.

Thompson's concept would lead to the idea of a portion of the labellum (the middle, No. 8) being part of the inner petal whorl while the lateral components form part of the staminal whorl. Gregory's (1936) interpretation is that the midrib portion is part of the outer-staminal whorl (along with the two staminodes) the inner whorl being made up of the two lateral components of the labellum and the fertile stamen. As has been shown already there is no clear evidence to regard the labellum as being made up of even two members, much less of three.

As regards the so-called stylar components, we have recognized only two glands. Even in Thompson's Fig. 103, only these two (18 and 19) are shown, the others are not shown in the serial transverse sections. Presumably they are emergences whose presence is implied rather than explicit. Even in the diagrammatic representation (Fig. 104) these two 18 and 19 are shown quite different from and bigger than the others (14–17). The reason for this is not clear. There is no evidence in this species of the presence of stylodes and we see no reason, so far as this material is concerned, to take up the view other than the one that has been elaborated, namely, that these are glands which, together with the fertile stamen, form the inner staminal whorl; the labellum is single which together with the staminodes which may or may not be present, form the outer androecial whorl.

VIII. Summary

The diploid chromosome number of *Alpinia calcarata* Rosc., is found to be 48.

Prochromosomes are present and their behaviour in mitosis is described. They are found to be the persistent chromatic portions adjacent to the centromeres. Their mitotic cycle is essentially the same as that of the normal chromosomes. There is no evidence to consider these as non-chromonematic.

The pollen tetrads are organized in a linear fashion. T-shaped tetrads are also common.

The embryo-sac develops in a normal manner from a hypodermal archesporium from which a primary parietal cell is cut off. There is usually only one layer of wall cells. The primary parietal cells do not usuall y undergo any further pariclinal division. Degeneration of the embryo-sac is met with very frequently and at all stages. Development beyond the 8-nucleate stage has not been met with. There is a haustorial-like attenuation of the antipodal end of the embryo-sac.

The ontogeny of the flower has been studied with special reference to the morphology of the labellum.

The labellum is considered to be a single structure and forms the anterior member of the outer staminal whorl, the two postero-lateral staminodes being absent. The inner staminal whorl is made up of the posterior fertile stamen and the two antero-lateral glands.

The frequent occurrence of flowers with two fertile stamens is interpreted on this basis.

The nature of the inflorescence is discussed and it is considered to be a scorpiod cyme.

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Plate XVII

Photomicrographs

Figs. 1–3.—Transverse sections of normal flower at successively higher levels from the top of the ovary. Note the appearance and disappearance of the ' glands ' in Figs. 1 and 2.

Fig. 3.—The two rounded staminode-like bodies are only the inrolled ends of the labellum.

Figs. 4–7.—Serial T.S. of two-stamened flower. Note that there is only one " gland", the other having become staminiferous. Note also that the second stamen is smaller, as also the labellum.

Fig. 8.—The anticlinal division of the primary parietal cell of the archesporium of the ovule.

Fig. 9.—Dyad Stage.

Fig. 10.—Mature embryo-sac with the haustorium-like antipodal end.

Fig. 11.—T.S. of a portion of the anther. Note the intrusive tapetum and 5 or 6 layers of wall cells.

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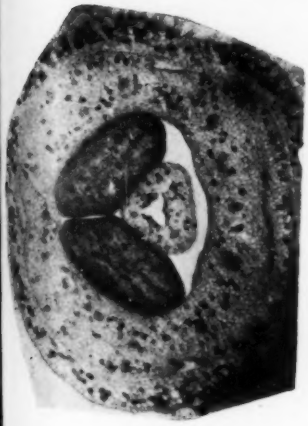

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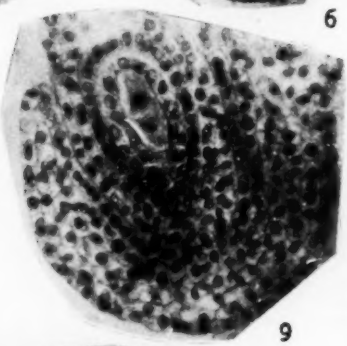

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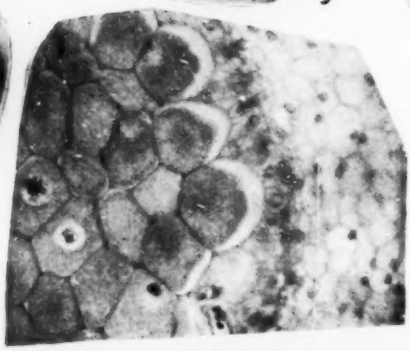

11

AMIDE SYNTHESIS IN PLANTS

I. The Succinoxidase System in Plants

By M. Damodaran and T. R. Venkatesan

(From the University Biochemical Laboratory, Chepauk, Madras)

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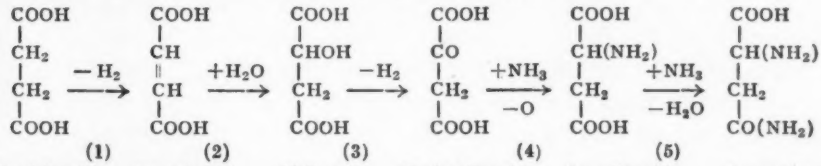
The probable reactions by which asparagine is formed in the plant are usually formulated according to the following scheme (*cf.* Chibnall, 1939)



Of the five steps involved, enzyme systems catalysing (3) and (5) are known to exist in plants. Malic dehydrogenase has been shown to be fairly widely distributed in the vegetable kingdom (Eüler, 1934) while asparaginase discovered by Grover & Chibnall (1927) in barley seedlings can be assumed to be capable of acting in reverse and converting aspartic acid into asparagine. On the other hand experimental findings regarding the existence of succinoxidase, the enzyme responsible for catalysing the first step in the scheme, have hitherto been negative in character. The position was summed up by Thunberg in 1938 in the following words:—" Es ist eine bemerkenswerte Tatsache, dass noch kein Samen entdeckt worden ist, der eine aktivierende Wirkung auf Bernsteintäure ausübt, während die Succinodehydrogenase zu den verbreitetsten und am stärksten wirkenden Dehydrogenasen in tierischen Geweben gehört."

Recently it was reported from this laboratory (Damodaran & Ramaswamy, 1940) that the reduction of methylene blue by extracts of certain seedlings was accelerated by succinic acid thus showing the presence in the extracts of succinic dehydrogenase. The present paper deals with experiments demonstrating the existence of the complete succinoxidase system in plants, its distribution in different species and a detailed description of the enzyme from one species, *Phaseolus mungo*.

Difficulty in demonstrating the presence of succinoxidase in plants arises from two factors. The activity of the enzyme is feeble compared to

that of other oxidases in the plant in contrast to animal tissues where it is among the most powerful of the enzymes present. Secondly the simple method of "washing" applied to muscle for removal of metabolites is not serviceable with minced plant material. A certain degree of purification and concentration is first essential before clear-cut results can be obtained either by the manometric or the Thunberg methylene blue technique. The preliminary experiments on methylene blue reduction already reported (Damodaran & Ramaswamy, *loc. cit*) were carried out with phosphate extracts of seedlings. It was found that differences in the times of decolorisation of methylene blue with and without succinic acid were small and not always reproducible. If however the phosphate extract is clarified by centrifuging at low speed and then brought to pH 4·7, the precipitate obtained is largely free from metabolites, has a low initial oxygen uptake and shows marked activity towards succinic acid both in methylene blue and manometric experiments.

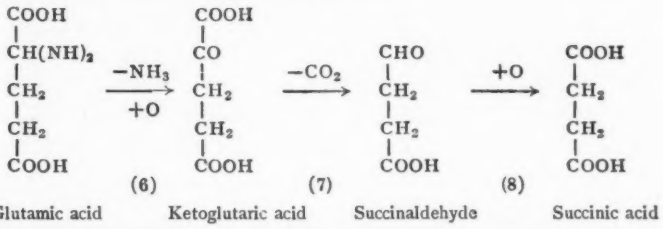
A detailed study of the enzyme preparation from two-day old seedlings of *P. mungo* shows that it corresponds in all respects to the succinoxidase from animal sources, being affected in the same way by inhibiting and accelerating agents. The oxygen uptake in the presence of succinate is reduced by about 60% by malonate (Quastel & Woolridge, 1926), 60% by pyrophosphate (Leloir & Dixon, 1937), 90% by oxalacetic acid (Das, 1937), 90% by persulphate (Keilin & Hartree, 1940) and completely inhibited by cyanide. An increase in activity of about 45% was obtained on addition of cytochrome. The optimal pH for the plant enzyme was however found to be 6·8 as against 7·3 to 7·4 for muscle succinoxidase.

Using the ingenious method of Stotz & Hastings (1937) who made a quantitative comparison of activity of the dehydrogenase and oxidase components of the system by measuring the oxygen uptake in the presence of cyanide and methylene blue it was found in agreement with the results obtained by these authors with muscle, that the dehydrogenase exerts only a part of the oxidising activity of the complete oxidase system. It is to be inferred from this experiment that the rate of reduction of methylene blue does not provide a true indication of the full activity of the enzyme which is attained only in conjunction with its natural hydrogen acceptor *viz.*, oxidised cytochrome [the presence of cytochrome in plants was demonstrated by Keilin (1925) and of cytochrome oxidase by Hill & Bhagavat (1939)]. It is also possible that the poisonous action of methylene blue on succinic dehydrogenase demonstrated by Yudkin (1933 and 1934) is a factor to be considered in this connection.

The end product of the reaction, fumaric acid, was determined by the method of Szent Györgyi & Straub (1935). Only about 60% of the amount

expected from the oxygen consumption was found, indicating that fumaric acid is probably further utilised in other reactions.

The distribution of the oxidase appears to be restricted both in regard to species as well as age of plants. Active succinoxidase is present in young seedlings (2–4 days old) as well as in the ripening pods of several members of the leguminosæ but not in the leaves or shoots of mature plants. In several other species examined, no activity could be detected at any stage of growth. Although there is a possibility that these negative findings may be due to the unsuitability of the same methods of concentration and purification for the enzyme from all sources, the results so far obtained make it clear that the universal or general occurence of succinoxidase in plants cannot be taken for granted. The presence of the enzyme in seedlings and pods of leguminous plants, where asparagine accumulation is known to be prominent supports the view that the carbon skeleton of the asparagine in plants is derived from succinic acid. As to the origin of the succinic acid itself we are at present completely in the dark. But there is some evidence that it can arise from glutamic acid by the following reactions :—



Carboxylase and aldehyde oxidase capable of bringing about reactions (7) and (8) have long been known in plants. More recently the existence of a glutamic dehydrogenase, catalysing reaction (6) has also been demonstrated in certain legumes (Damodaran & Nair, 1938). Further, analyses of certain species of seedlings have shown (Damodaran *et al.*, 1940) that during accumulation of asparagine the total dicarboxylic acid content remains unaltered indicating a conversion of glutamic to aspartic acid.

The suggestion has recently been made (Chibnall, *loc. cit.*; Vickery *et al.*, 1939) that succinic acid might play a role in plant respiration analogous to that postulated for it in animal tissues in the succinic acid cycle of Szent Györgyi and the citric acid cycle of Krebs. In both these theories succinoxidase occupies a central position. If, as our experiments indicate, the enzyme is present only in certain species and even in these only at certain stages of growth, it is difficult to see how the cycles of Szent Györgyi and Krebs can be operative in the respiration of plants. However, the possibility

that the negative results for succinoxidase obtained in certain cases may be due to unsuitable experimental conditions requires further examination.

Experimental

Methods.—The reduction of methylene blue in evacuated Thunberg tubes was employed for qualitative tests of dehydrogenase activity. Each tube as a rule contained 0·5 ml. of the extract to be tested, 0·2 ml. of M/2 succinate and 0·4 ml. of M/4,000 methylene blue in a total volume of 3 ml. of M/15 phosphate buffer. Oxygen uptake measurements were carried out in Warburg manometers. Except where otherwise stated the reaction mixture was made up as follows :—1·0 ml. enzyme, 0·25 ml. M/2 succinate and buffer to make up 2·6 ml.; 0·4 ml. of 20% KOH soaked on filter-paper was placed in the central cup for absorption of CO_2.

Preparation of active enzyme from Phaseolus mungo.—Two-day old seedlings germinated on sand were freed from testa, washed with distilled water and about 40 g. of the material thoroughly ground with gradual addition of 25 ml. of a well-cooled solution of M/10 Na_2HPO_4 in a granite mortar, previously cooled to 0°. The macerated material was centrifuged at 2,000 r.p.m. for 7 minutes for separation of inactive material. A thick scum formed on the top during centrifuging and it was found that sedimentation was better if this scum was scooped out carefully with a spatulla after the first minute or two of centrifugation. The turbid supernatant liquid which contained practically all the activity present in the original macerated material had a pH of 6·4–6·5, an initial oxygen uptake of 60–100 μl./ hr./ml. which more than doubled itself on addition of succinate.

Purification of the enzyme.—Several unsuccessful attempts were made before a more active preparation than the above extract could be obtained. Precipitation of the enzyme from the phosphate extract by half or full saturation with ammonium sulphate at various pH's gave preparations of low activity. Precipitation by alcohol and acetone also yielded inactive material. Grinding up of the seedlings with water followed by centrifuging at 10,000 r.p.m. and extraction of the residue with phosphate gave only a feebly active enzyme. The method which was finally found satisfactory was to bring an ice-cold solution of the phosphate extract (prepared as in the preceding paragraph) to pH 4·7 by careful addition of a 2–3% solution of acetic acid and to centrifuge immediately at 10,000 r.p.m. in ice-cooled tubes for 5 minutes. The supernatant possessed no activity and was discarded ; the compact brownish-yellow precipitate in the tubes was stirred up with ice-cold M/10 Na_2HPO_4 followed successively by M/4 and M/2 Na_2HPO_4 to raise the pH of the suspension to 6·8–6·9. The preparation from 40 g. of seedlings was usually dispersed in a total volume of 12–14 ml. of phosphate.

The colloidal solution thus obtained had a low initial oxygen uptake of less than 30 μl. which rose to 250–300 μl. per hour per ml. of enzyme on addition of succinate and could be preserved at 0° up to a week without loss of activity. Rise of temperature caused rapid inactivation which was complete in a few minutes at 50° C.

Variation of activity with pH.—The enzyme and substrate solutions were brought to the requisite pH by the addition of 1% acetic acid or M K_2HPO_4 as required and then buffered by means of Sørensen's phosphate solutions. In all cases the pH was taken both at the beginning and end of the experiments. The results (Table I, Figs. 1 and 2) show that the optimum has a narrow range

TABLE I

Effect of pH on Oxygen Uptake

Reaction Mixture—Enzyme 1·0 ml. succinate 0·25 ml. of M/2, buffer to make total volume to 2·6 ml.

	Oxygen uptake in μl. per ml. of enzyme							
*p*H of reaction mixture	5·0	6·2	6·45	6·7	6·8	7·0	7·2	8·4
30 minutes ..	41·5	87·5	112· 0	133·0	131·5	120·5	99·0	25
60 minutes ..	95·5	173·5	205· 5	244·5	239·0	223·5	158·0	37·5

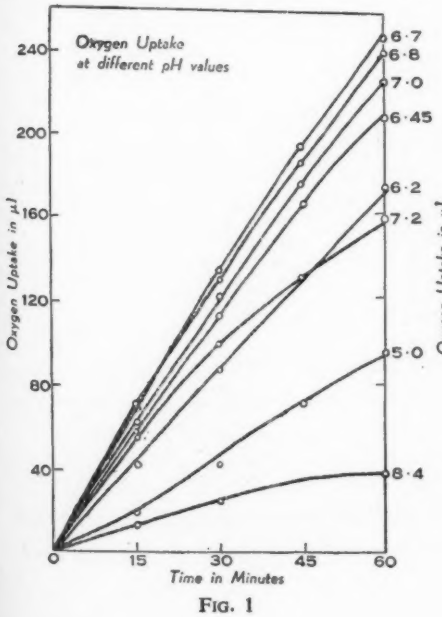


FIG. 1

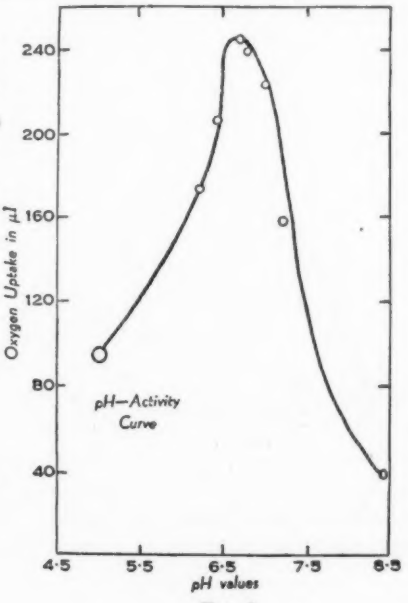


FIG. 2

lying between 6·6 and 6·9; the activity falls off more rapidly on the alkaline side than on the acid. In this respect the enzyme from vegetable sources shows a marked difference from muscle succinoxidase as the latter manifests its maximal activity and stability at a slightly alkaline reaction.

Activity and substrate concentration.—The variation of the rate of oxidation of succinate with its concentration is shown in Table II and Figs. 3 and 4,

TABLE II

Effect of Substrate Concentration on Oxygen Uptake

	Oxygen uptake in μl. per ml. of enzyme				
Concentration of succinate	0	M/100	M/50	M/25	M/12·5
30 minutes	15·0	64·5	89·0	95·0	80·0
60 minutes	26·0	101·0	144·5	157·5	138·5

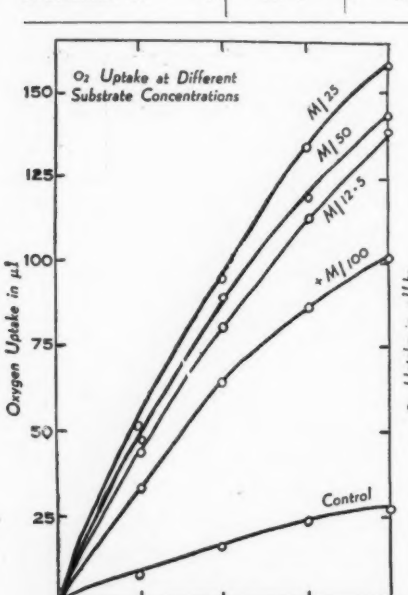


FIG. 3

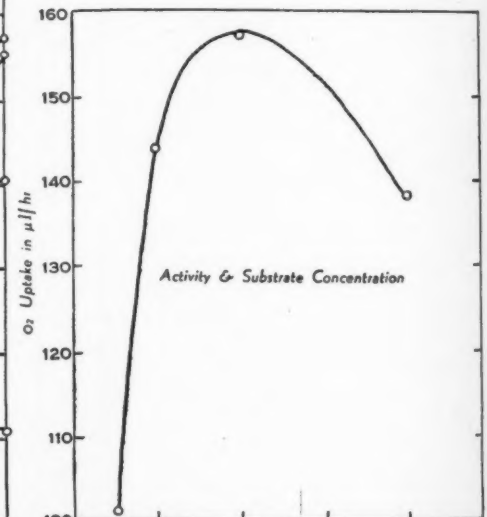


FIG. 4

with the enzyme preparation used in these experiments the optimum substrate concentration is seen to be about M/25.

Effect of inhibitors.—The effect of the following substances on oxygen consumption was determined: cyanide (Table III, Fig. 5), malonate (Table IV, Fig. 6) pyrophosphate (Table V), oxalacetic acid (Table VI), persulphate (Table VII), arsenite and fluoride. The last two substances had little or no effect in small concentrations. Cyanide and oxalacetic acid showed the most marked effects being able to suppress the oxygen uptake almost completely even at high dilutions. Malonate and pyrophosphate which are considered to be specific inhibitors of succinoxidase decreased oxygen uptake by about 70%, the effect being produced only at fairly high concentrations of the inhibitors. All the effects studied closely parallel to the results observed with the muscle enzyme. With pyrophosphate and oxalacetate it is very essential to employ solutions prepared immediately before use.

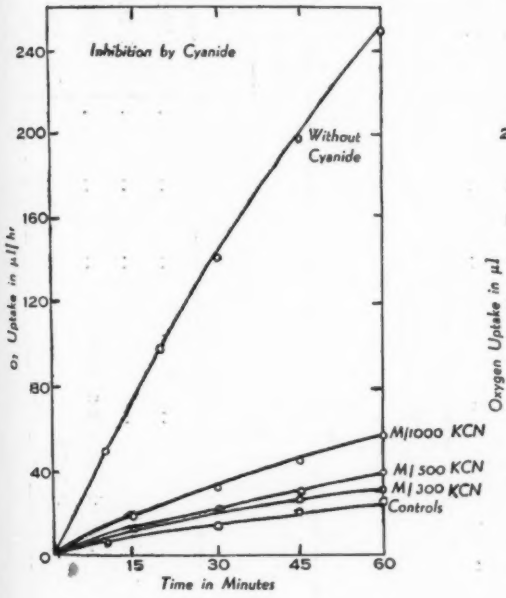


FIG. 5

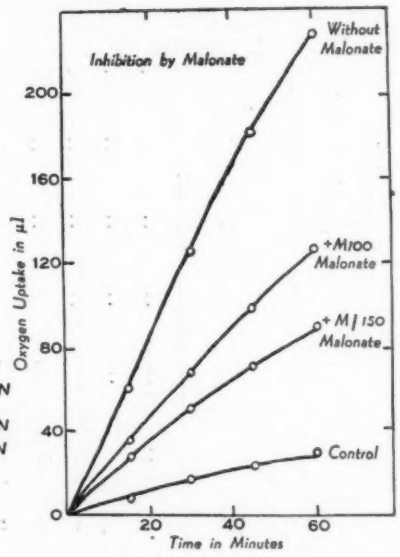


FIG. 6

Effect of cytochrome c.—Cytochrome *c* was prepared by the method of Keilin & Hartree (1937). Added to washed rat heart muscle the preparation produced a 100% increase in the oxygen uptake over that with succinate only. With the oxidase from *P. mungo*, the increase in activity was 40 to 50% (Table VIII, Fig. 7). The effect is much less than that noted by Ogston & Green (1935) with ox and sheep heart succinoxidase and by Elliott & Greig (1938) with the rat heart enzyme.

TABLE III

Inhibition by Cyanide

	Oxygen uptake in μl. per ml. of enzyme		% Inhibition in 1 hour
	30 minutes	60 minutes	
Enzyme 1·0 ml.	13·4	25·7	
„ „ + M/25 succinate	141·5	249·0	
„ „ „ „ + M/1000 cyanide	31·5	57·2	86·0
„ „ „ „ + M/500 „	20·9	39·6	94·2
„ „ „ „ + M/300 „	20·4	30·6	97·8

TABLE IV

Inhibition by Malonate

	Oxygen uptake in μl. per ml. of enzyme		% Inhibition in 1 hour
	30 minutes	60 minutes	
Enzyme 1·0 ml.	16·5	29·1	
„ „ + M/25 succinate	125·7	229·4	
„ „ „ „ + M/100 malonate	68·0	127·6	50·2
„ „ „ „ + M/50 „	51·8	91·2	69·0

TABLE V

TABLE V

Inhibition by Pyrophosphate

	Oxygen uptake in μl. per ml. of enzyme		% Inhibition in 1 hour
	30 minutes	60 minutes	
Enzyme 1·0 ml.	15·3	25·8	
„ „ + M/25 succinate	139·6	232·9	
„ „ „ „ + M/100 Pyrophosphate	70·6	126·5	51·0
„ „ „ „ + M/50 „	64·9	111·8	58·5
„ „ „ „ + M/25 „	49·3	87·3	70·3

TABLE VI

Inhibition by Oxalacetic Acid

	Oxygen uptake in μl. per ml. of enzyme		% Inhibition in 1 hour
	30 minutes	60 minutes	
Enzyme 1·0 ml.	11·5	20·9	
„ „ + M/25 succinate	87·5	166·9	
„ „ „ „ + M/2000 Oxalacetic acid	20·3	36·6	89·2

TABLE VII

Inhibition by Persulphate

	Oxygen uptake in μl. per ml. of enzyme		% Inhibition in 1 hour
	30 minutes	60 minutes	
Enzyme 1·0 ml.	9·2	16·8	
„ „ + M/25 succinate	60·1	107·5	
„ „ „ „ + M/2000 persulphate	16·4	29·0	89·6

TABLE VIII

Effect of Cytochrome c

	Oxygen uptake in μl. per ml. of enzyme		% Increase in 1 hour
	30 minutes	60 minutes	
Enzyme 1·0 ml.	11·5	21·0	
„ „ + M/25 succinate	77·5	152·5	
„ „ „ „ + 0·2 ml. cytochrome	101·5	186·1	25·6
„ „ „ „ + 0·4 ml. „	111·9	209·5	43·4
„ „ „ „ + 1·0 ml. „	114·1	215·2	46·7

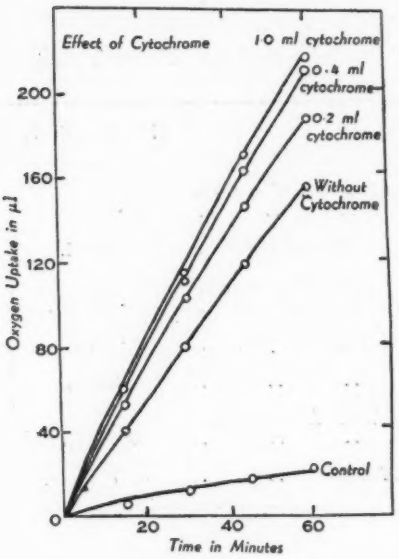


FIG. 7

Determination of fumaric acid.—Estimations of fumaric acid were carried out both in the control and the experimental solutions immediately after the oxygen uptake measurements were completed by the method of Szent Györgyi & Straub (*loc. cit.*). The enzyme was inactivated and protein precipitated from the reaction mixture by addition of one-fourth the volume of 20% trichloracetic acid, the precipitate centrifuged off and washed with 4% trichloracetic acid, supernatant and washings combined and aliquots extracted three times with pure ether distilled over alkaline permanganate. The ether was evaporated off on the water-bath after addition of a small amount of water to the extract and the aqueous residue titrated in acid medium against N/100 potassium permanganate. A great disadvantage of the method is the loss of fumaric acid by adsorption on protein during precipitation by trichloracetic acid. Corrections for this loss were applied as suggested by Szent Györgyi & Straub; even so, only 60% of the fumaric acid (Table IX) calculated from oxygen uptake could be accounted for. Stotz (1937) in his experiments, found only a third of the fumaric acid expected.

TABLE IX

Formation of Fumaric Acid

	without succinate	with M/25 succinate
Oxygen uptake in μl. per 1·25 ml. of enzyme per hour	41·5	315.2
Volume of N/100 $KMnO_4$ used for titration ..	0·78 ml.	7·20 ml.
Weight of fumaric acid present (1·16 mg. of fumaric acid = 6·14 ml. of N/100 $KMnO_4$) ..	0·140 mg.	1·295 mg.
Correction of fumaric acid lost by adsorption ..	0·094 „	0·698 „
Total fumaric acid found	0·234 „	1·993 „
Theoretical amount calculated from oxygen uptake	0·420 „	3·265 „
Percentage of fumaric acid found	55·7	61·0

Determination of the activities of the dehydrogenase and the oxidase components of the enzyme.—Manometric measurement of the dehydrogenase activity was carried out by complete inhibition of the oxidase component with M/200 potassium cyanide and studying the oxygen uptake in presence of methylene blue in a concentration of M/1000 as carrier (Stotz & Hastings, *loc. cit.*); cytochrome oxidase activity was determined by measuring oxygen uptake in presence of dimethyl *p*-phenylenediamine (Stotz & Hastings, *loc. cit.*; Elliott & Greig, 1938) in place of succinate. It was found (Table X, Fig. 8,) that the oxidase component is considerably more active than the dehydrogenase and that the oxygen consumption in the presence of methylene blue is only about 10–15% of that of the complete succinoxidase system. By the same method the dehydrogenase in animal tissues was found to be 30–50% of the total succinoxidase activity (Stotz & Hastings, *loc. cit.*; Keilin, 1940).

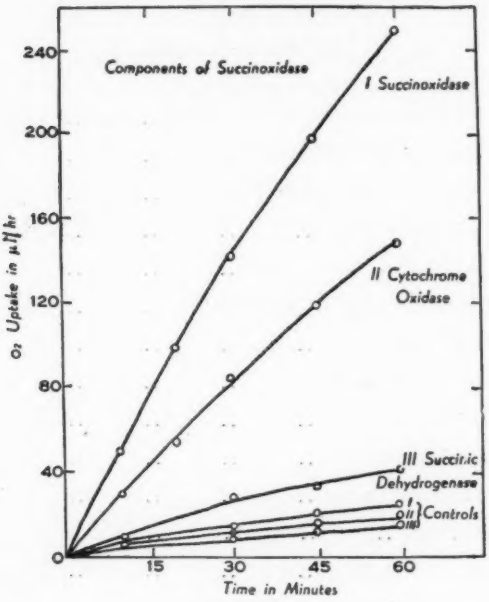


FIG. 8

TABLE X

Components of Succinoxidase

(I and II refer to separate Experiments)

		Oxygen uptake in μl. per hour per ml. of enzyme
Succinoxidase (1 ml. enzyme and M/25 succinate) ..	I	223·3
	II	157·6
Succinic dehydrogenase (1 ml. enzyme, M/25 succinate, M/200 KCN and M/1000 methylene blue)	I	22·9
	II	22·6
Cytochrome oxidase (1 ml. enzyme, M/30 dimethyl *p*-phenylenediamine)	I	124·5
	II	75·4

Occurrence of succinoxidase.—Oxidase and dehydrogenase activity was determined on phosphate extracts of macerated plant material and on the precipitate obtained on acidifying such extracts to pH 4·7 as described for the preparation of the enzyme from *P. mungo*. The results are summarised in Table XI.

TABLE XI

The Distribution of Succinoxidase

Phaseolus mungo seedlings (2–3 days)	+ + +
„ „ leaves of mature plants	−
„ „ shoots of mature plants	−
„ „ Ripening pods	+ +
Dolichos biflorus seedlings (2–3 days)	+ +
„ „ leaves and shoots of mature plants	−
Cicer arietinum seedlings (2–4 days)	+ +
Pisum sativum seedlings (2 days old)	+ +
Dolichos lablab tender pods	+
„ „ ripening pods	+ +
Vigna catiang seedlings (3 days old)	+ +
„ „ mature pods	+ +
Oryza sativa seedlings (4–6 days old)	−
Pennisetum typhoideum seedlings (2 days old)	+
Andropogon sorghum seedlings (3 days old)	+
Musa paradisiaca tender shoots	−
Commelina benghalensis tender shoots	−
Allium cepa tuber slices (7 days old)	−
„ „ shoot slices (7 days old)	−
Citrullus vulgaris seedlings (4 days old)	−
Luffa ægyptiaca tender shoots and leaves	−
Bougainvillea tender shoots	−
Acalypta sandliana tender shoots	−

Summary

The presence of succinic dehydrogenase and oxidase has been demonstrated in young seedlings and pods of certain leguminosæ. The enzyme could not be detected in leaves and shoots of mature plants of the same species nor in seedlings of certain other species.

A highly active preparation of the enzyme has been made from seedlings of *Phaseolus mungo*. A detailed study shows that the properties and behaviour

of this enzyme are in all respects analogus to those for muscle succin-oxidase.

The presence of the enzyme in seedlings and pods of leguminosæ where asparagine formation is known to be predominant, lends support to the view that succinic acid is a precursor of asparagine in plants.

In view of the restricted occurrence of the enzyme, it is premature to postulate the existence of the succinate and citrate cycles in plant respiration.

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THE DEVELOPMENT OF THE EMBRYO SAC IN *VOGELIA INDICA*, LAMK.

By Kanhaiyalal Mathur, M.Sc.
(*Government College, Ajmer*)
AND
Reayat Khan, M.Sc.
(*Department of Biology, Dacca University*)

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1. Introduction

Since the publication of Dahlgren's monograph in 1916, some very important work has been done during recent years on the Plumbaginaceæ. We are now led to recognise at least five different types of embryo sac development in this family :—

1. *Penaea*-type.—This type has so far been described only in one species belonging to Plumbaginaceæ, *viz.*, *Statice Eu-Limonium* (Fagerlind, 1938*b*). The four megaspore nuclei, unseparated by walls, are arranged in a crosswise fashion and by two further divisions give rise to four quartets of parietally placed nuclei; one nucleus from each quartet migrates towards the centre to form a tetraploid secondary nucleus. The remaining three nuclei of each quartet develop walls around them but the micropylar triad alone functions as an egg apparatus.

2. *Plumbago*-type.—In this type the four megaspore nuclei, unseparated by walls, divide only once resulting in four parietally situated pairs of nuclei. One nucleus from each pair migrates to the centre, all the four fusing to form a tetraploid secondary nucleus. The nucleus left at the micropylar end then organises into the egg cell and that at the chalazal end forms a single antipodal which soon degenerates. The two lateral nuclei also become cut off as cells, sometimes becoming egg-like but usually soon disappearing so that the mature embryo sac possesses only two nuclei—the egg and the secondary nucleus. Such a type was first reported by Haupt (1934) in *Plumbago capensis* and later in *P. zeylanica* (Dahlgren, 1937), *P. scandens* (Boyes, unpublished), *P. coccinea* (Boyes, unpublished), *Ceratostigma plumbaginoides* (Dahlgren, 1937) and *C. willmottianum* (Boyes, unpublished).

3. *Fritillaria*-type.—This type has recently been reported in *Armeria bupleuroides* (Fagerlind, 1938*b*). Here the egg is removed from the megaspore mother cell nucleus by four divisions. Three out of the four megaspore nuclei migrate towards the chalazal end and fuse and the third division results in a secondary 4-nucleate stage. The fourth division produces eight

nuclei which become organised into the mature embryo sac as in *Fritillaria* or *Lilium*.

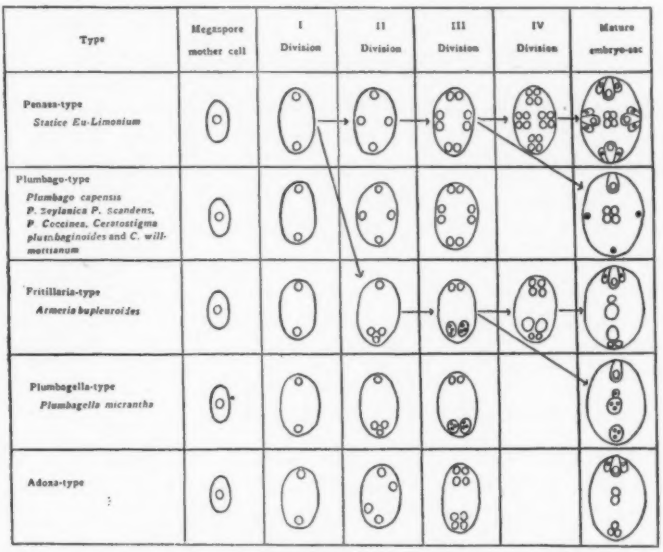


FIG. 1. Types of embryo sac development in Plumbaginaceæ. The arrows indicate their probable phylogenetic relationships (Diagrammatic).

4. *Plumbagella*–type.—Dahlgren's account of the embryo sac development in *Plumbagella micrantha* has been shown to be incorrect and a new type of development is now described for this plant (Fagerlind, 1938 *a* and Boyes, 1939 *a* and *b*). As in *Fritillaria*, three of the four megaspore nuclei migrate to the chalazal end and fuse producing a secondary 2-nucleate stage. The two nuclei then divide producing the secondary 4-nucleate stage with two haploid nuclei at the micropylar end and two triploid ones at the chalazal end. No further divisions occur. The secondary nucleus is tetraploid, being formed by the union of one haploid and one triploid nucleus; the egg is haploid and the single antipodal is triploid. The mature embryo sac, after polar fusion and disappearance of the antipodal cell, shows only two nuclei.

5. *Adoxa*–type.—This was reported by Dahlgren in eleven different species of the Staticeæ, belonging to the genera *Armeria* and *Statice*. In some cases this has been definitely shown to be incorrect ; a reinvestigation of all the others is desirable. It is indeed doubtful if the *Adoxa*-type occurs at all in the Plumbaginaceæ.

All these types are represented diagrammatically in Fig. 1 which also indicates their probable phylogenetic relationships except that of the *Adoxa*-

type the occurrence of which in the Plumbaginaceæ is already considered doubtful. *Vogelia indica* conforms to the *Plumbago*-type as will be evident from the description that follows.

2. *Material and Methods*

Vogelia indica is a stout perennial shrub, 4 to 5 feet in height, with several branches arising from the base and spreading out above. The leaves are very leathery and xerophytic ; the flowers appear after the rains in the cold season and the red coloured spikes are then very prominent. It grows on rocky hills at Ajmer and Mt. Abu in Rajputana. According to Duthie (1911) the genus has only three species with a very restricted distribution, one belonging to S. Africa and the other two to Arabia; of the latter, one extends to India, thereby suggesting that this genus is an old one, phylogenetically much nearer to its ancestral stock. A similar claim has been made for *Statice* (Maury, 1886) and *Plumbagella* (Boyes, 1939). The comparative antiquity of the plant will be considered later.

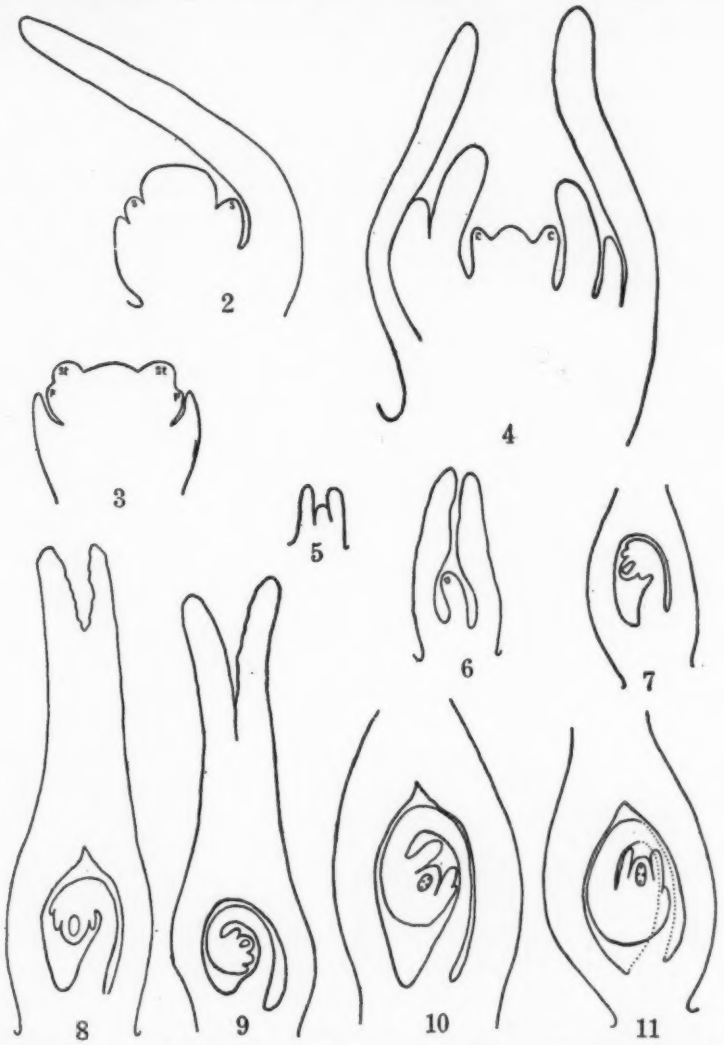
The material was fixed in FAA and Nawaschin's fluid under an exhaust pump. In the case of younger buds the winged calyx was removed before fixing; in older flowers the ovaries could be dissected out before fixation. Sections were cut at 6–10 microns for younger stages and 12–15 microns for mature embryo sacs. Iron-alum Hæmatoxylin was used for staining according to the method described by Maheshwari (1938 *a*).

3. *Organogeny*

The origin of the floral parts takes place in the usual acropetal succession—sepals, petals, stamens and carpels. Fig. 2 illustrates the origin of sepals and Fig. 3 that of the petals as well as the stamens. The carpels arise as an annular outgrowth round the apical region (Fig. 4) the latter forming directly the ovule which is thus of cauline origin. The further growth and ultimate fusion of the carpellary annular outgrowth are illustrated by Figs. 5, 6 and 7. The style is solid and develops feathery appendages on its lower portion.

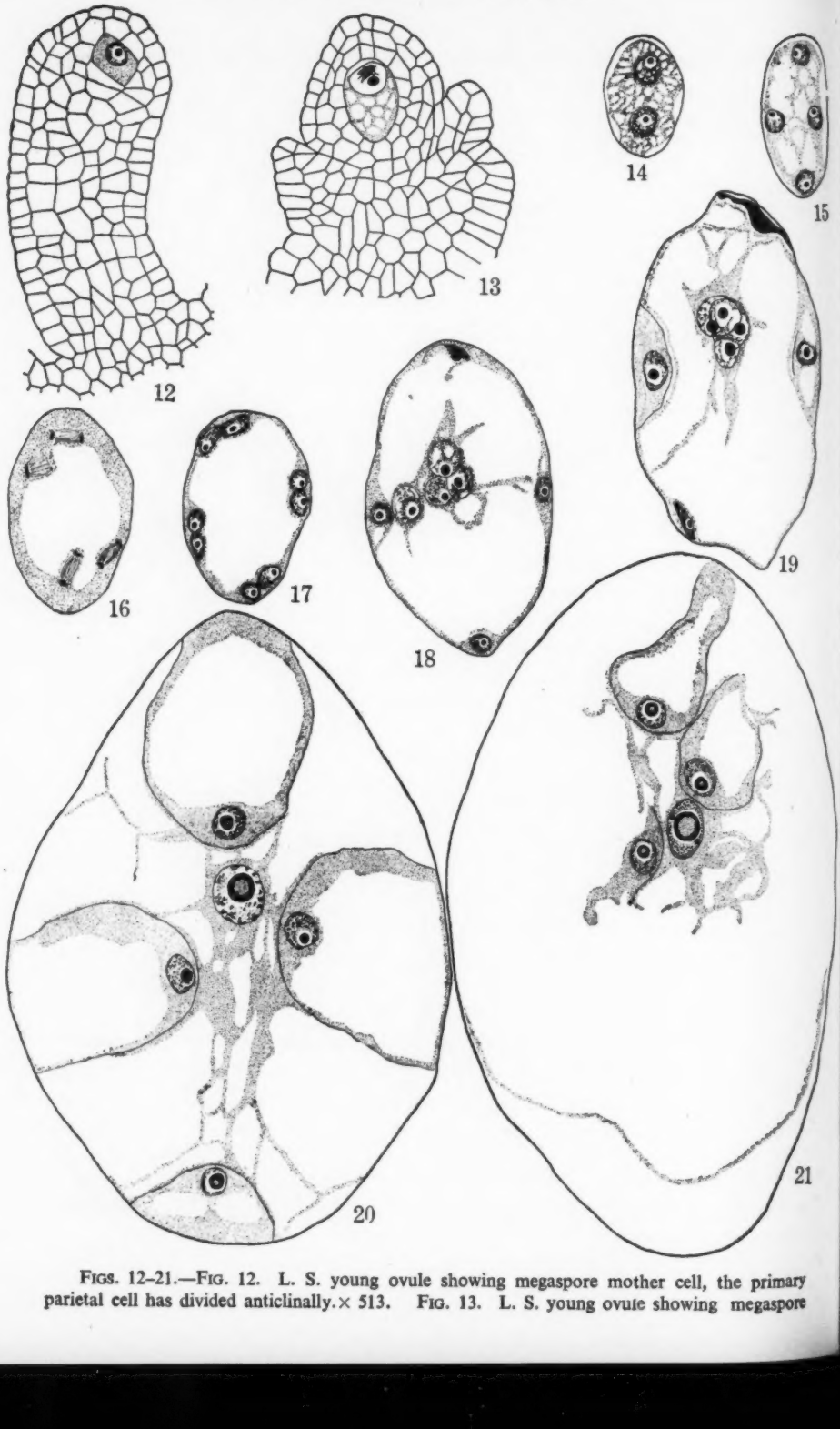
4. *Ovule*

The growth and inversion of the single, cauline ovule (Figs. 5–8) take place in a manner essentially similar to that described by Haupt (1934) for *Plumbago capensis*. The subsequent turning up (Figs. 9–11) is very rapid and by the time the embryo sac is mature, the micropylar region of the ovule again points upward as it did when the ovule had just been laid down (Fig. 11). This is due to the rapid elongation of the funicle which shows greater growth on its outer side than the inner, thus leading to the continued curvature of



FIGS. 2–11. FIG. 2. Origin of sepals (S) ×148. FIG. 3. Origin of petals (P) and stamens. (St.) × 148. FIG. 4. The carpellary outgrowth (C) around the apical region which will develop into the ovule. × 148. FIG. 5. Next stage in the development of the carpels and the ovule ×79. FIG. 6. Carpellary outgrowth a little before fusion; the ovule is slightly bent towards one side. × 79. FIG. 7. The upper portion of the carpellary outgrowth has fused forming the solid style; the ovule shows a still greater curvature. × 79. FIGS. 8–11. Further stages in the curvature of the ovule which finally points upwards once again. × 79.

the ovule. There are two integuments (Fig. 13) a feature that *Vogelia* possesses in common with other Plumbaginaceæ.



FIGS. 12–21.—FIG. 12. L. S. young ovule showing megaspore mother cell, the primary parietal cell has divided anticlinally. × 513. FIG. 13. L. S. young ovule showing megaspore

mother cell in synizesis, 2–3 layered tapetum and the rudiments of the integuments. × 513. FIG. 14. Binucleate embryo sac. × 513. FIG. 15. Four megaspore nuclei arranged crosswise. × 513. FIG. 16. The four megaspore nuclei dividing simultaneously; the cytoplasm has become restricted to the peripheral region of the embryo sac. ×513. FIG. 17. Four pairs of parietally placed nuclei. × 513. FIG. 18. One nucleus from each pair migrating towards the centre; three of them have already reached their destination. × 513. FIG. 19. Three nuclei in the centre, one of them is binucleolate and has been produced by the fusion of two; the micropylar nucleus has degenerated and the chalazal is also degenerating. ×513. FIG. 20. The tetraploid secondary nucleus formed after fusion, the micropylar cell organised into the egg, the lateral cells enlarged and egg like; the chalazal cell, though smaller than the others, has also enlarged. × 513. FIG. 21. Mature embryo sac with the egg and the secondary nucleus; the lateral cells also are present but the chalazal one has completely disappeared. × 513.

5. *The Archesporium*

There is a single hypodermal archesporial cell arising before the origin of the integuments and at a time when the ovular protuberance is still upright. By a periclinal wall it soon divides into an outer primary parietal cell and an inner megaspore mother cell (Fig. 12). The former divides anticlinally into two cells (Fig. 12) which by further divisions give rise to two to three layers of parietal tissue (Fig. 13). The presence of such a tissue in this sympetalous family is rather significant. This and the Cucurbitaceæ both have been well known for the wall-formation in their nucelli which are thus quite broad and do not correspond to the slender type seen in other sympetalean families. Among the higher, sympetalous families there are only a few cases so far reported of the formation of wall cells, notably in the Convolvulaceæ (see Fedortschuk, 1931 ; Mathur, 1934).

More than one megaspore mother cell was never observed in the same ovule although in *Plumbago capensis* the presence of two megaspore mother cells in the same nucellus is of such frequent occurrence that it can hardly be called an abnormality (Haupt, 1934).

6. *Formation of megaspore nuclei and embryo sac*

The nucleus of the megaspore mother cell enters the synizesis stage and undergoes reduction division to form four megaspore nuclei. Fig. 14 shows the 2-nucleate embryo sac with only small vacuoles at this stage. In the 4-nucleate stage (Fig. 15) vacuolation becomes more prominent and the four nuclei become arranged in a crosswise manner. These now divide simultaneously (Fig. 16) forming four pairs, all parietally placed owing to the formation of a large central vacuole (Fig. 17).

7. *Organisation of the mature embryo sac*

The organisation of the mature embryo sac is very peculiar and closely resembles that in *Plumbago capensis* (Haupt, 1934). One nucleus from each

of the four pairs of nuclei migrates towards the centre and all the four meet a little below the egg, giving rise to a single tetraploid secondary nucleus (Figs. 18, 19 and 20). It was noted that all the four nuclei may not fuse simultaneously but by stages. Thus, Fig. 19 shows that two nuclei have already fused forming a binucleolate nucleus while the other two still retain their individuality. Of the remaining four nuclei at the periphery the one at the micropylar end exhibits a remarkable increase in size and becomes organized into the egg cell, being separated from the cavity of the embryo sac by a membrane. The nucleus is situated against the basal portion of the membrane, the greater part of the cell being occupied by a single large vacuole (Figs. 20 and 21). The nucleus at the chalazal end becomes organised into a single antipodal which soon degenerates and is usually not to be found in the mature embryo sac. The two lateral cells also are delimited by membranes and generally disappear, although sometimes they may enlarge and assume an egg-like appearance, protruding to a considerable extent towards the centre (Fig. 20). In *Plumbago capensis* (Haupt, 1934) occasionally even the antipodal cell becomes enlarged and egg-like. The synergids are absent and in one or two cases the micropylar cell that usually becomes the egg was seen degenerating (Figs. 18 and 19).

Details of fertilisation were not available in the material that was sectioned, but some stages of embryonal development have been observed. In all cases only a single micropylar embryo was seen; and the possibility of poly-embryony expected on account of the egg-like appearance of lateral cells was not confirmed. Thus these cells do not seem to function as eggs except in very exceptional circumstances.

8. Discussion

The embryo sac of *Vogelia* like that of *Plumbago* and *Ceratostigma*, is tetrasporic, tetrapolar and 8-nucleate; the egg being removed from the megaspore mother cell by three divisions. In these respects it resembles the *Adoxa*-type. But in the matter of organisation of the mature embryo sac there is a closer resemblance with the *Penaea*-type, especially in the formation of a tetraploid secondary nucleus and in the frequent presence of the egg-like lateral cells. The important difference from the *Penaea*-type lies in the omission of the fourth division, which also accounts for the absence of the synergids in *Vogelia*. It thus appears that the type of the embryo sac in *Vogelia* can be easily derived from the *Penaea*-type simply by the elimination of the fourth division with its necessary consequences. This view has been expressed both by Haupt (1934) and Boyes (1939) and Fagerlind is also in agreement with them. The fact that a tetrasporic and tetrapolar 16-nucleate embryo

sac also occurs in this family at least in one species of *Statice* (Fagerlind, 1938 *b*) lends further support to this view.

A comparison with *Acalypha indica* (Maheshwari and Johri, 1940) is very illuminating, because the embryo sac resembles the *Penaea*-type on the one hand and the *Plumbago* on the other. In all these three embryo sacs the fundamental basis for the organisation of the mature embryo sac seems to be the same, and the method of migration and disposal of the free nuclei is also similar with the only difference that it begins in *Vogelia* just after the third division, while in the other two it is postponed till after the fourth division and hence the difference also in the number of nuclei involved in this disposal at the four poles and the centre. It will be pertinent to point out in this connection that the family Euphorbiaceæ like Plumbaginaceæ is also noted for showing considerable variation in the development of the embryo sac (see Maheshwari, 1937, for details), and it seems that these plants possess a greater latitude and freedom from the normal course in the matter of organisation of their embryo sacs, the phylogenetic or evolutionary significance of which is still shrouded in mystery.

With regard to the family Plumbaginaceæ it is suggested that of the five types of embryo sacs outlined in Fig. 1, the *Penaea*-type is the most primitive, the others may all be derived directly from it except *Plumbagella* which is more closely related to the *Fritillaria*-type seen in *Armeria bupleuroides*.

9. *Summary*

(1) The floral parts develop in the usual acropetal succession.

(2) Usually a single hypodermal archesporial cell is differentiated in the nucellus. This divides to form a primary wall cell and a megaspore mother cell.

(3) The primary wall cell undergoes a few periclinal and anticlinal divisions giving rise to two or three layers of parietal tissue.

(4) No tetrad of spores is formed, the megaspore mother cell directly developing into the embryo sac.

(5) As a result of the meiotic divisions the nucleus of the mother cell produces 4 nuclei which become arranged in a cross-wise fashion. Each of these nuclei divides once resulting in 4 peripherally placed pairs of nuclei, while the centre is occupied by a large vacuole. One of the two micropylar nuclei is organised as the egg, one nucleus from each pair migrates towards the centre and all the four fuse together to form a tetraploid secondary nucleus. Of the three nuclei, which are left over, the one at the chalazal

end quickly degenerates, while those at the sides often enlarge and assume an egg-like appearance.

10. Acknowledgement

In conclusion we have great pleasure in expressing our sincere thanks to Dr. P. Maheshwari of the Dacca University at whose suggestion the work was started and who has very kindly favoured us with his helpful suggestions and criticisms.

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STUDIES ON THE HELMINTH PARASITES OF KASHMIR

Part I. Description of Some New Species of the Genus, *Pomphorhynchus* Monticelli (1905)

By B. L. Kaw, M.Sc.

Lecturer in Biology, S. P. College, Srinagar, Kashmir
(From the Department of Zoology, The University, Lucknow)

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Monticelli (1905) erected the genus *Pomphorhynchus* to include *Echinorhynchus lævis*, *Echinorhynchus tereticollis*, and *Echinorhynchus proteus*. He based his diagnosis on the presence of a characteristic spherical swelling at the anterior end of the neck. Porta (1907) gave a complete diagnosis of the genus and took *Pomphorhynchus proteus* as the type species. Linstow (1908) described another species from Turkestan and called it *P. perforator*. Van Cleave (1919–24) described the species *P. bulbocolli*, from North American fishes and further held that *Pomphorhynchus lævis* (Syn. *Echinorhynchus lævis*) was the valid name for the species *P. proteus* and therefore, on grounds of priority, it should be used in place of *P. proteus*. Mayer (1933) raised the genus *Pomphorhynchus* to the status of a subfamily Pomphorhynchinæ, which is represented by a single genus consisting of only three species so far reported from Europe, North America, Asia Minor and Turkestan. Specimens described in the present communication were collected during the early summer of 1938 from Srinagar and adjoining places in Kashmir. They include two forms, one from the intestine of a Cyprinoid fish (*Nemachilus kashmirensis*) and the other from the sub-peritoneal region of a frog (*Rana cyanophlyctis*). In the latter case only the juvenile or unripe forms were recovered. The present collection is rather interesting as the genus *Pomphorhynchus* is being reported for the first time from India. Moreover, the juvenile forms of *Pomphorhynchus* so far known only in Salmonids and smaller Cyprinoids are now recorded from frogs.

This work was carried out at the University of Lucknow, under the guidance of Dr. G. S. Thapar to whom the author is greatly indebted for many valuable suggestions and also for permission to use his personal library. The author is also thankful to Dr. M. B. Lal for advice and revision of the

manuscript. Dr. S. L. Hora of the Indian Museum was very kind to identify the hosts, for which the author feels deeply indebted to him.

Pomphorhynchus kashmirensis n.sp.

A number of specimens of this species were collected from the intestine of a fish, *Nemachilus kashmirensis*, in Kashmir and since they differ from all the known species of the genus *Pomphorhynchus* they are being described as a new species.

The body presents three marked regions—proboscis, neck and trunk. The males which are smaller than the females in size, are 10·3 to 11·6 mm. long; the females are 12·7 mm. in length. Proboscis (Fig. 3) is cylindrical, 0·55 to 0·62 mm. long and 0·25 to 0·36 mm. in diameter. It is beset with thorn-shaped hooks arranged in radial longitudinal rows and these alternate in the adjacent rows. The number of longitudinal rows in this species is 14–16 with 11–12 hooks in each row. The hind-most hooks of the longitudinal rows disturb the normal arrangement and lie in a single horizontal line. The hooks slightly increase in length from before backwards, but become more slender and less curved, so that the posterior ones appear to stand out sharply from the body. The root is indistinctly forked in the anterior hooks. The last horizontal row of hooks shows only thin projections but no backwardly directed root. The dimensions of the various hooks in the longitudinal rows have been measured as follows :—

Hook No. in longitudinal row	Length of hook	Breadth at base	Length of root	Form of root
3rd	0·024	0·011	0·039	Slightly forked.
5th	0·022	0·017	0·042	Not forked.
7th (*a*)	0·023	0·07	0·029	Not forked.
7th (*b*) ..	0·028	0·08	0·031	Not forked.
9th	0·028	0·005	0·022	Not forked, with projection at the top.
11th	0·041	0·005	..	Small projection.

The proboscis is followed by an elongated cylindrical neck, which, towards the proboscis end, is dilated into a balloon-like 'bulla'. Neck, excluding the bulla, is 0·9 mm. to 1·05 mm. long with a diameter of 0·25 to 0·5 mm. The spherical bulla extends over 0·65 to 1·3 mm. of the neck and has its diameter 0·65 to 1·5 mm. The trunk is elongated, cylindrical and tapers

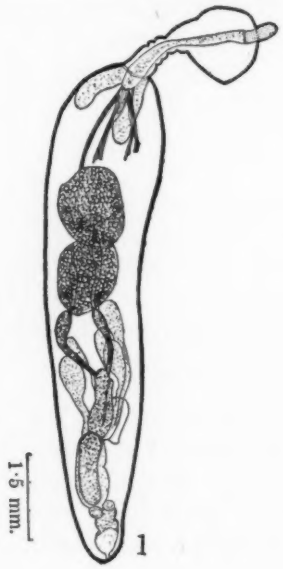
towards the posterior end. It is 8·5 to 10 mm. long and its maximum diameter which lies in the anterior half of the body is 1·9 to 2·5 mm.

The outer layer of the body-wall consists of a tough and thick elastic cuticle, underneath which lies the subcuticular layer consisting of a large number of scattered nuclei but the cell limits are not clearly seen. Beneath the subcuticular layer lies the musculature of the body consisting of an outer layer of circular and an inner layer of longitudinal muscle fibres.

The Lacunar system (Fig. 3) consists of two main lateral longitudinal vessels running throughout the length of the body. These unite with each other by a network of transverse vessels, all of which are full of granular fluid.

There is a large body cavity surrounding the various internal organs. The cavity of the ' bulla ' is in communication with that of the trunk by a canal in the neck. This is further verified from a study of the gravid females in which the eggs have travelled into the cavity of the bulla.

Proboscis receptacle is a sac-like structure, greatly elongated, about 2·2 to 2·8 mm. long and extends from the posterior end of the proboscis through the neck and hangs inside the anterior part of the trunk cavity. It is double-walled but the outer wall seems incomplete inasmuch as it does not cover the receptacle posteriorly. There are two sets of muscles—the



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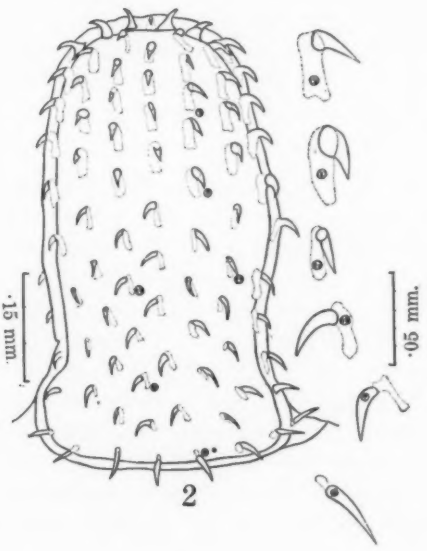

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inverters and the retractors—that control the movements of the proboscis and are attached to the body wall.

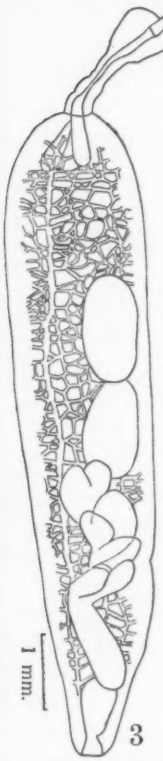
Brain consists of a ganglionated-mass about ·095 mm. long and ·065 mm. broad and lies in the posterior part of the proboscis receptacle at a distance of about 0·15 mm. from its base.

Lemnisci are two elongated cylindrical structures, 0·8–1·35 × 0·26–0·45 mm. in dimensions, lying in the anterior part of the trunk cavity lateral to the receptacle. Each lemniscus consists of a syncytial mass of tissue with large number of nuclei.

Male reproductive system (Figs. 1, 3 and 4) consists of two testes and their ducts together with certain accessory structures and penis that are described below. The two testes are more or less globular in shape and lie in tandem in the body cavity at about 1/5 of the body length of the animal from the anterior end. They are approximately equal in size, the anterior testis is 1·4 × 1 mm. and the posterior testis 1·3 × 1 mm. The two vasa deferentia arising from the testes enlarge into three pairs of seminal vesicles (*i.e.*, vesicula seminales) in the sexually mature specimens and unite to form a common duct within the genital shield. A small genital ganglion is seen lying posteriorly on one side of this shield. Prostate glands are multinucleate, globular to pear-shaped structures, 0·65–0·95 × 0·3–0·45 mm. and vary in number from five to eight. Prostate ducts unite with the common vas deferens to form ductus-ejaculatorius. Behind this lies the bell-shaped bursa copulatrix, which possesses anteriorly two lateral bursal pouches between which lies the penis. The penis is slightly oval, ·12 mm. long and ·08 mm. broad, and in this the ejaculatory duct opens. The gential shield contains in addition to the vasa deferentia and the ejaculatory duct, an elongated muscle sac forming the muscle pouch.

The female specimen (Fig. 5) is gravid and as the whole of its body cavity including the neck and the 'bulla' is full of eggs, the various internal organs are masked and can only be located with difficulty. The female reproductive system, however, consists of egg-balls, uterine bell, uterus and vagina. Ovary is broken up into large number of egg-balls which are held up by the genital ligament. The uterine bell is present and as its name signifies it is more or less bell-like, and bears posteriorly the dorsal opening. Uterus is an elongated tube, 1·4 mm. long and 0·12 to 0·17 mm. in diameter. Its wall is fairly thick and bears muscular band outside. There is no ovejector but the uterus becomes slightly thickened in the middle. Vagina represents the last part and leads to the exterior at the female genital pore. It is 0·29 mm. long and 0·1 mm. in diameter with an external opening guarded by a

muscular sphincter. At the posterior end it bears the protractor muscle which connects it to the musculature of the body wall. Eggs are thick-shelled and contain developing embryos and fill up both the uterus and the vagina.



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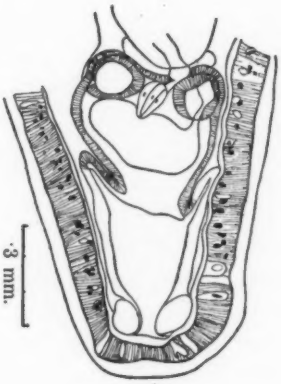

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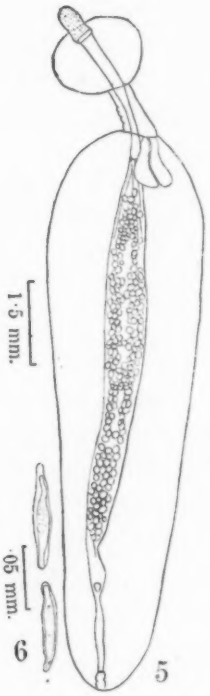

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An embryo is more or less spindle-shaped, ·075— ·085 mm. long and ·01–·017 mm. in diameter.

Discussion

The present form differs from other known species in number and arrangement of hooks—a character which has been considered of great taxonomic importance in the determination of the various species of the genus *Pomphorhynchus*.

As regards the validity of *P. proteus* and *P. tereticollis* as independent species it appears that both these forms show the same number and arrangement of the hooks as are found in *P. lævis*, *i.e.*, there are 18-20 longitudinal rows of 11–13 hooks and since this is the only character for the determination of the species of the genus, the author considers them all to come under

the same specific name. *P. lævis* having priority over others is, therefore, retained and others are considered as synonyms to it. The following key is given for the determination of the species of the genus, *Pomphorhynchus*.

Key to various species.—

Proboscis hooks:

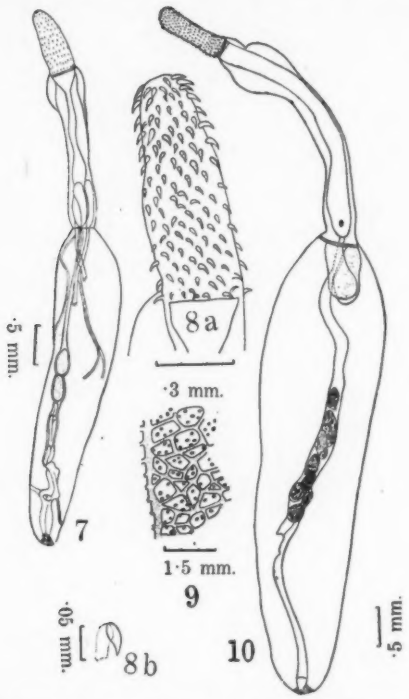
12 longitudinal rows with 12 to 14 hooks in each row	*P. bulbocolli.*
16 longitudinal rows with 10 hooks in each row	*P. perforator.*
18 to 20 longitudinal rows with 11 to 13 hooks in each row	*P. lævis.*
14 to 16 longitudinal rows with 11 to 12 hooks in each row	*P. kashmirensis* (n. sp.)

A Juvenile Form of *Pomphorhynchus* from the Frog, *Rana cyanophlyctis*

This form was obtained from the sub-peritoneal region of the local frog, *Rana cyanophlyctis*, at Srinagar in Kashmir. The collection includes both male and female specimens but they are young forms, not fully mature. In general anatomy they resemble the genus *Pomphorhynchus*.

Males (Fig. 7) are smaller than the females, being 6·9 to 7·1 mm. long. The females (Fig. 10) are 8·75–10·3 mm. in length. The body is divisible into three parts—proboscis, neck and trunk. A thin cuticular ingrowth is present between neck and trunk. Proboscis is cylindrical, 0·75–1 mm. long and 0·21–0·28 mm. in diameter ; its length being three to four times that of its diameter. Neck is cylindrical and elongated, 1·95–3·3 mm. long (about $\frac{1}{4}$ to $\frac{1}{3}$ of the body length) and 0·3–0·4 mm. broad. The relative size of both proboscis and neck is longer than that of *P. kashmirensis*. 'Bulla' is less marked and has a diameter of 0·5–0·7 mm. Trunk is of a uniform thickness for a greater part of its length and is cylindrical. Proboscis hooks are arranged in 18 longitudinal rows with 13 hooks in each row. In this character it resembles *Pomphorhynchus lævis* and differs from all other known species of the genus *Pomphorhynchus*. The hooks of the adjacent rows alternate with each other, except the basal ones that are arranged in a single horizontal line. A hook in the middle region of the proboscis is ·095 mm. long and its root which is more or less undivided is ·12 mm. long.

Body-wall has a thick cuticle on the outside, below which lies the syncytial tissue with large number of nuclei.



Lacunar system consists of two lateral longitudinal vessels with a close anastomosis of branches.

The body-wall musculature lies below subcuticular layer.

The proboscis receptacle is an elongated tubular and double-walled structure lying only in the neck region and not extending to the cavity of the trunk. The outer wall or sheath is incomplete, and thus resembles the previous species. The genital ligament arises from the posterior end of receptacle and extends into the body cavity surrounding the genital organs. There are two retractors and four invertors of the proboscis connecting it with the body-wall.

Brain is a ganglionated mass, ·1–·11 mm. long and ·07–·08 mm. broad and lies at a distance of ·135–·16 mm. from the base of the inner sheath of the receptacle. It is here comparatively larger than in the previous species. It gives off in front two anterior nerves and on the sides two bands of nerve-fibres.

There are two lemnisci and as in other species, each consists of a syncytial mass of tissue with a large number of nuclei. They are flattened and variable

in form and position. They lie either protruding in neck or hanging in the cavity of trunk. Sometimes an intermediate arrangement is seen with one lemniscus lying in the neck and the other in the trunk. Lemnisci are generally elongated but sometimes shorter and broader (0·6–0·9 mm. × 0·2–0·4 mm.).

Male reproductive system is simpler than in the other species, certain parts being not developed. There are two small testes equal in size (0·37 × 0·2 mm. each) hanging by the genital ligament in the body cavity, and these lie one behind the other. No seminal vesicles have been seen as these organs only develop at the time of sexual maturity. Prostate glands are 6 in number and comparatively small and bear large number of nuclei. Prostate ducts unite with the vasa deferentia to form ejaculatory duct. This is followed by a muscular elongated and tubular bursa, 0·65 × 0·18 mm. in size. Anteteriorly it bears two pouches and posteriorly a wide opening. It is attached to the body-wall by two sets of muscles—protractor and retractor. Penis lies anteriorly between two bursal pouches and is an elongate structure, ·07 mm. × ·02 mm.

The female reproductive system (Fig. 10) consists of an ovary divided into egg-balls which are held together in genital ligament. No eggs or embryos are seen. Uterine bell is present and it bears a posterior dorsal opening. Uterus is a fairly elongated tube-like structure, 1·7 mm. long and 0·1 mm. in diameter. Its wall is thick and contains muscular band outside. In one specimen it was seen to possess two swellings in the middle separated by a narrow thin portion. Vagina is 0·2 mm. long and 0·13 mm. broad and bears on the outside a sphincter. On the inside it has elongated dumb-bell-shaped cells with nuclei at their posterior ends. There is a protractor muscle attached at its posterior end.

The specimens belong to the genus *Pomphorhynchus*. The male and female reproductive organs are not fully developed and no eggs or embryos are seen in its body cavity. It posesses certain peculiarities which are to a large extent like those found in the juvenile forms of *Pomphorhynchus lævis*—trunk is cylindrical, bulla is not much prominent, receptacle does not descend in the trunk cavity and lemnisci are variable in form and position.

Among the known species of the genus, juvenile forms of *P. lævis* have been found to occur in *Salmo fario* and in *Salmo saler* and they are reported even without ' bulla '. *P. lævis* also shows change of hosts and the smaller Cyprinoids are said to play the part of intermediate hosts. Siebold about eighty years ago, mentioned a crustacean, *Gammarus pulex*, as one of the intermediate hosts, but this has not been confirmed.

The present form resembles *P. lævis* in the number of hooks on proboscis and juvenile character, but as *P. lævis* has not so far been reported from India and is never said to exist in juvenile stage in frog, it is doubtful, whether the form described above can be treated as the juvenile stage of *P. lævis*. It is therefore tentatively kept as a new species under the name *Pomphorhynchus dubious*.

EXPLANATION OF FIGURES

Pomphorhynchus kashmirensis (n. sp.)

FIG. 1. Male specimen.
FIG. 2. Proboscis and proboscis hooks.
FIG. 3. Lacunar system.
FIG. 4. Posterior region of male specimen (magnified).
FIG. 5. Female specimen.
FIG. 6. Eggs.

Pomphorhynchus dubious (n. sp.?)

FIG. 7. Male specimen.
FIG. 8 (*a*). Proboscis, and
(*b*). Proboscis hook.
FIG. 9. Portion of lacunar system.
FIG. 10. Female specimen.

(Type specimens are deposited in Dr. Thapar's Helminthological collections, Lucknow University.)

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THE SPERMATOGENESIS OF *CLIBANARIUS OLIVACEOUS*, HENDERSON

By Miss C. K. Rathnavathy, M.Sc.

(From the University Zoology Research Laboratory, Madras)

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Introduction

DESPITE extensive work done in the field of Crustacean spermatogenesis a great deal of controversy still exists regarding the mode of formation of the various parts of the non-flagellate sperm. It is therefore the object of this study to trace the origin and development of the various components, both nuclear and cytoplasmic, in the radiate type of sperm of the decapod, *Clibanarius olivaceous*.

The species occurs on the east coast of India and has been recorded in the Chilka lake. The form is abundant in the backwaters of Adyar, and the individuals seek retreat in the empty shells of the gastropod, *Potamides*. The males are slightly larger than the females and the form breeds practically throughout the year. The work was done mainly between August 1936 and July 1937.

Previous Work

As early as 1874 Hallez published a note on the development of the spermatozooids in Brachyuran Decapods and a little later Grobben (1878) made a study of the male reproductive organs of the Decapods. In 1885 Sabatier worked on the spermatogenesis of the decapod crustacea, and a similar study was made by Herrmann in 1883. Grobben and Sabatier paid particular attention to the origin of the spermatogonia, but Gilson in 1886 made a comparative study of the spermatogenesis of the Arthropods and concentrated on the development of the spermatogonia and the metamorphosis of the spermatid into the spermatozoon. Herrmann in 1890 studied the structure and development of the spermatozooids of the Decapoda. Still later in 1902 a study of the spermatogenesis of *Oniscus acellus* was taken up by Nichols, special attention being paid to the history of the chromatin. Labbe's interesting contributions, one on the spermatogenesis of the decapod crustacea and another on the maturation of the spermatids and the constitution of the spermatoozoids of the decapod crustacea in the years 1903 and 1904 respectively deal with the behaviour of the cytoplasmic inclusion like the mitochondria besides the activities of the nucleus. Andrews studied " Crayfish spermatozoa" in 1904 and in 1906 Grobben published his observations on the Decapod sperms under the title " Zur kenntnis der Decapoden Spermien". In the same year Keppen (1906) recorded his observations on the male germinal cells of *Astacus fluviatilis*, followed, three years later, by " Comparative studies in Crustacean spermatogenesis " (1909) by Nichols. In the same year Koltzoff (1909) studied decapoden spermatogenesis while Retzius (1909) made a study of the Crustacean sperms and Spitschakoff (1909) published his paper entitled " Spermien und spermiohistogenese bei

Cariden". Binford's account of "The germ cells and the process of fertilization in the crab, *Menippe mercenaria*" in 1913 provides us with a knowledge of the nuclear changes that occur in spermatogenesis, and also deals with the activities of the mitochondria. In the same year Reinhard (1913) published "Zum bau der spermien und zur spermatogenese von *Potamobius leptodactylus* (*Astacus leptodactylus*)". Nathan Fasten added to our knowledge in this field by his accounts of the spermatogenesis of the American crayfish *Cambarus virilis* and *Cambarus immunis* (1914) and another on *Cancer magister* (1918). In 1921 Fasten studied "The explosion of the spermatozoa of the crab, *Lophopanopeus bellus*" and in 1924 he published the "Comparative stages in the spermatogenesis of various Cancer crabs". Two years hence (1926) appeared the "Spermatogenesis of the black-clawed crab, *Lophopanopeus bellus*". Coincident with this publication Susaeta (1926) presented her observations on the bursting of the spermatozooids of the Decapod Crustacea and Vejdovsk'y (1926) his researches on the "Spermatogenesis of the Crayfish". In this connection I may mention the very valuable researches of Grabowska on the cytoplasmic constituents in the male germ cells of *Astacus fluviatilis* and *Astacus leptodactylus* in 1927 and of *Potamobius astacus* in 1929. Baker studied the spermatogenesis in *Branchipus vernalis* and published a series of papers between the years 1927 and 1928.

As late as 1932 Vishwa Nath studied "The spermatid and the sperm in *Paratelphusa spinigera*," and as no previous work has dealt with the formation of the acrosome in the crustacean sperm, I think it would be worth while here to give a brief summary of his observations on the form.

The earliest spermatid has a perfectly spherical nucleus with a faintly staining medullary region and a darker cortical one. In the cytoplasm could be distinguished a darkly staining granule—the centrosome, extremely delicate scattered vesicles—the mitochondria, and lastly a few Golgi bodies lying in the form of rings near the nucleus.

Four changes concerning these four structures take place now inside the spermatid. The nucleus begins to stain darkly and uniformly, the centrosome becomes rod-shaped and then divides into-two ; the mitochondria grow and become more resistant to acetic acid, larger vesicles being gradually formed by the running together of the smaller ones, and the Golgi rings come to be arranged closer together and begin to stain more densely. Eventually the Golgi bodies form a single mass which is destined to form the acrosome and corresponds to the "mitochondria-like" mass of Fasten.

The mitochondrial vesicles when they have run together result in a large clear vesicle, corresponding to the primary vacuole of Fasten, and

simultaneous with the completion of this vesicle the two centrosome place themselves near the base of the vesicle, one of the two soon after, establishing itself on what will become the distal border of the vesicle. This distal centrosome soon becomes ring like—the so-called chromatin ring of Fasten. The nucleus becomes flattened and then cup-shaped, the mitochondrial vesicle fitting into the cavity of the nuclear cup. The acrosome lies on the rim of the nuclear cup. The proximal centrosome which was hitherto a granule grows into a small vesicle and corresponds to the secondary vesicle of Fasten.

The acrosome next expands into a band, gets converted into a ring and fuses with the margin of the nuclear cup thus forming the nuclear-acrosomal cup which corresponds to the nuclear-mitochondrial cup of Fasten. At this time the axial filament, corresponding to the central body of Fasten, grows from the bottom of the proximal centrosome and after piercing it and the mitochondrial vesicle stops just below the distal centrosome. The central body shows a transverse piece at its distal end which fits into the middle of the ring-like distal centrosome serving to keep the mitochondrial vesicle well pressed within the nuclear-acrosomal cup.

The process of spermioteleosis is thus completed. The spermatozoon when looked at from the bottom appears as a disc, the margin of the disc staining deeply and representing the fused nucleus and acrosome. Within this is the very faintly staining mitochondrial vesicle in the centre of which lies the vesicular proximal centrosome containing the darkly staining axial filament.

Dealing with the formation of the acrosome, Nath says that the acrosome is produced as a result of the direct transformation of the Golgi and not as a result of any secretory activity on the part of the Golgi, no mention being made of a Golgi remnant or remnant of the acroblast anywhere in his observations. Reviewing Bowen's studies on the spermatid cells of the Hemiptera (1922 *a*) it is found that he depicts the existence of a Golgi remnant which is left in the cytoplasm of the spermatid after the secretion and deposition of the acrosome by the acroblast, this Golgi remnant or remnant of the acroblast being said to be later sloughed off from the body of the cell when the sperm is reaching maturity. This secretion of the acrosome by the acroblast and the later sloughing off of the Golgi remnant Bowen describes as of general occurrence in the spermatogenesis of Insects. Elaboration on this point is reserved to the section dealing with a discussion on the subject.

Since the publication of Nath's work on *Paratelphusa spinigera* Muthuswamy Iyer (1933) published a paper on the spermatogenesis of *Paratelphusa hydrodromus*. This last mentioned account deals with the changes

concerning both the nuclear and cytoplasmic structures. The mitochondria are here described as small vesicles which later run in together to form a distinct big vacuole and this corresponds to the primary vacuole of Fasten. The acrosome is first described as a deeply-staining compact body in which could be made out ring-like structures and this later develops into the ring-like acrosome. Here again the acrosome is represented as being formed by the direct transformation of the Golgi substance, mention not being made about the existence either of an acrosomal vesicle or of a Golgi remnant.

Two other works of importance that may be mentioned in connection with Decapodan Spermatogenesis are those by Vishwa Nath of which one was published in the year 1937 while only an abstract of the other was published in 1938. In his paper of 1937 on the spermatogenesis of *Palæmon lamarrei*, Nath describes the degeneration of Golgi during spermioteleosis, and in 1938 he arrives at the same conclusion after his observations on the two hermit crabs, *Cœnobita* and *Clibanarius longitarsus*. He moreover, describes the existence of a centrosome in the spermatid cells of *Cœnobita* and *Clibanarius* which he says remains single and does not divide during spermioteleosis unlike those in *Paratelphusa* (1932) and *Palæmon* (1937) where division of the original centrosome into proximal and distal centrosomes takes place.

The above is a brief review of almost all the important contributions so far made towards the study of the spermatogenesis of the decapod crustacea and it will be seen that only a very few authors have paid any attention to the cytoplasmic elements of the germ cells of the Crustaceans. Of these Vishwa Nath (1932) and Muthuswamy Iyer (1933) are the only authors who have gone so far as to trace the formation of the acrosome in the two Decapods, *Paratelphusa spinigera* and *Paratelphusa hydrodromus*, and the spermatogenesis of this Anomuran, *Clibanarius* has been chosen as the subject of investigation in order to make a thorough study of both the nuclear and cytoplasmic components of its germ cells.

Since the present work was completed there appeared a paper by Worley entitled " A study of the sperm-forming components in three species of Decapoda," wherein she deals with the activities of the three cytoplasmic constituents, the golgi, mitochondria and centrosome in great detail. On reviewing this paper the results of which seem to differ in certain vital respects from my observations on *Clibanarius*, another decapod species, I have found it necessary to add a note in discussion at the end of my paper which I hope will help to clarify the results that I have obtained from my researches on the present form.

Material and Methods

The material used for this investigation was obtained in plenty from the backwaters of Adyar. The testes were dissected in normal saline solution and after being completely cleansed of their surrounding tissue immediately transferred to the various fixatives. The fixatives employed were:—

Corrosive-sublimate acetic,
Bouin's fluid,
Carnoy's fluid,
Flemming with acetic,
Flemming without acetic,
Champy's fluid,
Mann-Kopsch's fluid and its modification by Ludford,
Kolatchev's fluid,
Nassanov's fluid and
Da Fano's fluid.

Of these Carnoy's fluid and Bouin's fluid were found to be very useful for chromosomal work, as also Flemming's fluid with the addition of acetic acid. The last mentioned fixative was found to give excellent pictures of the nucleus especially those of the spindle with the centrosomes at the polar ends. Flemming without acetic was found to demonstrate the acrosome and the Golgi remnant in a distinctive manner and Champy's fluid to fix the cytoplasmic elements very clearly, the mitochondria standing out in a remarkable manner when stained with iron hæmatoxylin. The methods of Champy-Kull and Benda were also attempted with fairly good success. For the detection of Golgi bodies Mann-Kopsch's fluid and its modification by Ludford were found to be indispensable. An immersion for about five days in 2% osmic acid was necessary to bring about the Golgi elements distinctly, the blackening from the mitochondria being very easily extracted by immersion in turpentine. Nassanov's modification of Kolatchev's fluid was found to demonstrate the acrosome in a distinctive manner.

Paraffin method of embedding was employed in all cases and sections were cut varying in thickness from four to ten microns. Of these, sections cut between seven and ten microns were found to be the most useful for nuclear study while the rest were found necessary for the study of the cytoplasmic constituents.

Iron hæmatoxylin was found in all cases to be the most useful stain for work on chromosomes, Bordeaux red, van Geison, and Eosin being sometimes used as counter stains. Delafield's hæmatoxylin was also tried. Champy-

Kull stain was used in some cases after Champy fixation. Benda's Alizarin method gave fairly good results. Altmann's stain after Mann-Kopsch fixation gave satisfactory results. Intravitam staining with Janus green and Neutral red did not yield very good results. Smears also were extensively used.

The Male Reproductive System

The testis (Fig. 1) of *Clibanarius* is an elongated paired tubular organ lying just underneath the dorsal wall of the abdomen and extending from the anterior end of the abdomen to about three quarters the length of the same to the posterior end. The inner edges of the right and left halves lie close together, the whole structure being partially enveloped by fatty tissue. The testis is narrow at the anterior extremity and wide posteriorly and is composed of a number of follicles each made up of long tubules which vary in diameter. The convoluted vasa deferentia originate posteriorly and diverging laterally run anteriorly and open ventrally at the base of the last pair of thoracic legs of the animal. The gonadial tube along its course shows extreme convolutions and forms several complete loops before it reaches the exterior. It is transparent and through its walls may be seen packets of sperms or spermatophores arranged in a linear order on peaked processes of a transparent basal membrane. The membrane and sperm sacs are formed as a secretion product of the layer of epithelium that lines the vas deferens.

The Testicular Follicles

The walls of the testicular follicles are thin and contain small, rounded or oval deeply-staining nuclei. When sections of follicles (Fig. 2) are examined a number of well-defined tubules may be distinguished in one follicle. For instance, on one side of the sectioned follicle there may be a number of tubules of spermatogonia and the rest of the follicle may contain either transforming spermatids or primary spermatocyte divisions or divisions of the secondary spermatocyte stage or even mature sperms. Not only may the seminal elements in the separate parallel cavities of a follicle differ in the stages of their development, but in the same tubule the elements at one end of it may be further advanced in their development than those at the other end. Thus in the cavity at one end of a tubule the cells may be in the early prophase of the division of the primary spermatocytes, while at the other end they may have reached the spermatid stage. All the stages in the transformation of the spermatid into the spermatozoon may be found in passing from one end of a tubule to the other. When the younger generations of germ cells mature, the walls separating groups of such cells rupture so that maturing sperms lie in a common cavity. It thus follows that at the very posterior end of the testis the sperms lie in a common cavity leading to the vas deferens on each side.

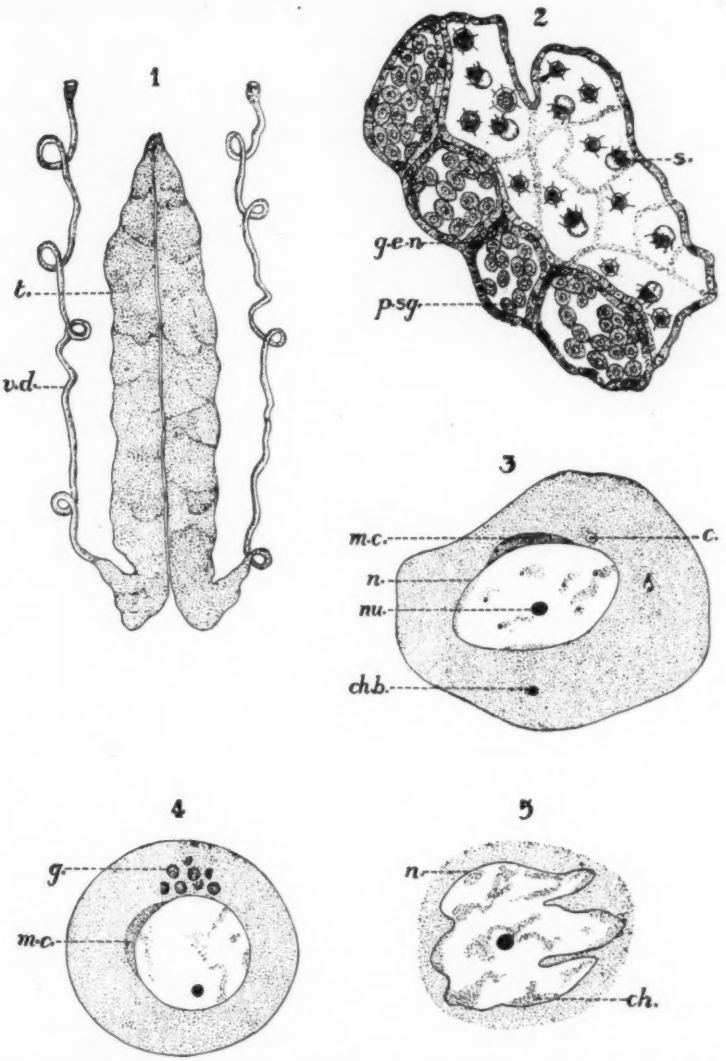
The Spermatogonial Stages

When a section of a testicular follicle (Fig. 2) is examined, it is found to be composed of a number of tubules, each tubule being lined by the germinal epithelium which is of the nature of a syncitium. The nuclei of the germinal epithelium are large and contain numerous irregular clumps of chromatin the whole taking on a distinctive deep blue colour when stained with iron hæmatoxylin. These nuclei grow rapidly and become spherical and soon get surrounded by a distinct mass of cytoplasm thus forming the spermatogonia.

The resting primary spermatogonium (Fig. 3) has a large spherical or ovoid nucleus with a distinct nucleolus which has been identified as a result of chromatin tests, to be of the nature of a karyosome. Chromatin granules, of varying size are distributed throughout the nucleus. In the cytoplasm could be distinguished darkly-staining bodies which could be identified as chromatoid bodies. They may be spheroidal, elongated or irregular in shape and stain as intensely as chromatin with basic dyes. Some of them are so large as to be easily recognizable under the low power of the microscope while others are extremely minute. The centrosome is a single minute spherical body lying close to the nucleus. These two structures are perfectly visible in material fixed in nuclear fixatives such as Carnoy, Bouin, Corrosive-sublimate-acetic etc., and stained by iron hæmatoxylin. In Champy-fixed material however could be distinguished lying in the cytoplasm and adjacent to the nucleus, a small deeply-staining crescentic mass which is of the nature of mitochondria (Fig. 3 and Photograph 1). Besides this structure in Mann-Kopsch preparations, small ring-like or crescent-shaped discrete bodies could be distinguished in the cytoplasm, these being of the nature of golgi bodies (Fig. 4). But for the presence of the above-mentioned structures, the cytoplasm has a more or less uniform appearance throughout and each spermatogonium is surrounded by a fairly definite and distinct cytoplasmic wall which distinguishes it from its fellows.

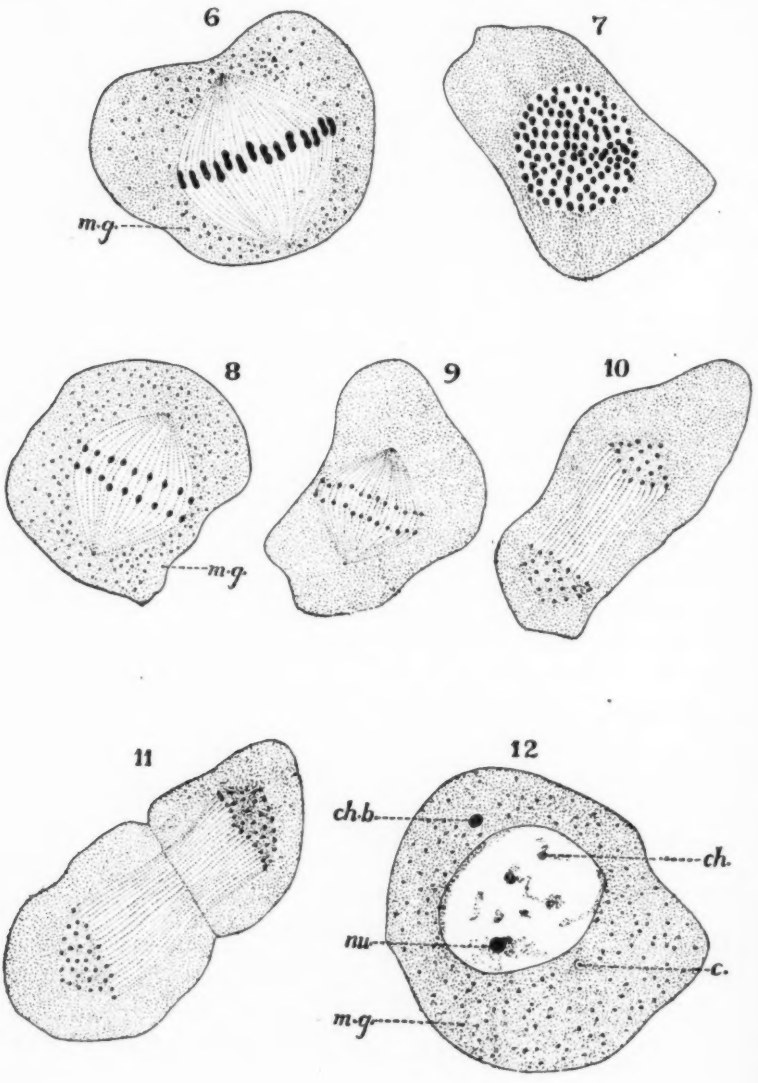
Interspersed between the spermatogonial cells are certain cells with deeply-staining irregular nuclei. These are the nutritive cells (Fig. 5). They bear some resemblance to the spermatogonial cells but may be distinguished by the shape of their nuclei being elongated, crescentic or triangular or even with pseudopodia-like processes and also by the fact that their nuclei stain darker than those of the surrounding cells. Their mode of division is highly suggestive of amitosis and their constant occurrence among the spermatogonia implies a probable origin from the spermatogonial cells.

When the primary spermatogonium begins to divide, the chromatin clumps assume the form of a large number of spheres which migrate towards



the centre of the nucleus. Meanwhile the centrosome divides, and the two products of division travel to the opposite poles of the cell between which the spindle is formed. With the formation of the spindle the nuclear wall gets disorganized. The chromosomes in the equatorial plate are dumb-bell-shaped and the spindle fibres delicate in appearance (Fig. 6). In polar views of sections of the equatorial plate, the chromosomes are observed to be

distributed throughout the whole plane of the equator (Fig. 7). They are small and spherical in appearance and a hundred and sixteen chromosomes can be counted. With the division of the primary spermatogonia the Golgi elements increase in number and get scattered in the cytoplasm. The mitochondrial mass also breaks up into small granules which get scattered in the substance



of the cytoplasm (Figs. 6 and 8). The anaphase (Figs. 8, 9 and 10) and telophase (Fig. 11) stages follow each other quickly thus dividing the cell into secondary spermatogonia. During the division of the primary spermatogonia the Golgi bodies and mitochondria get distributed approximately to the two resulting cells. The secondary spermatogonia are slightly smaller than the primary spermatogonia and the chromatin in their nuclei stains much more heavily. The ultimate divisions of the secondary spermatogonia produce resting primary spermatocytes.

The Primary Spermatocyte Stage

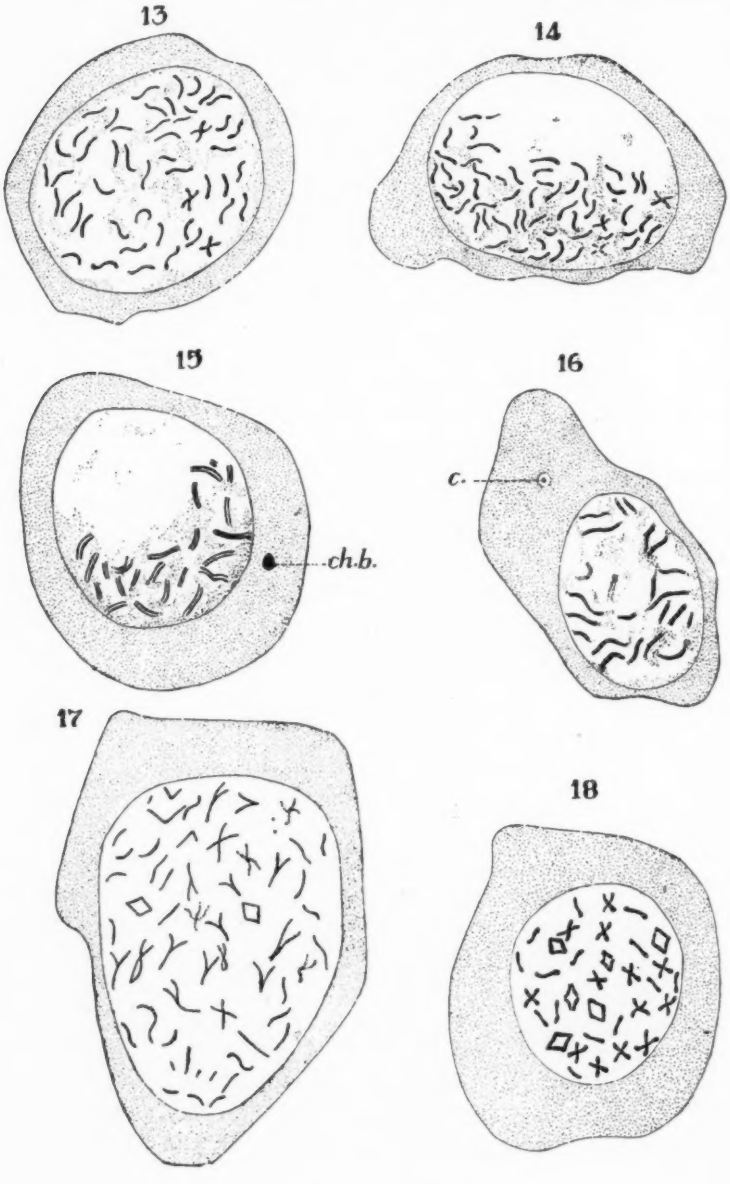
The primary spermatocyte (Fig. 12) is a fairly large cell and within its nucleus may be distinguished indiscriminately scattered chromatin granules. The nucleolus is a prominent structure and is usually excentric in position although occasionally it is seen to lie in the centre of the nucleus.

In the cytoplasm, the centrosome may be distinguished as a minute, deeply staining granule. Huge chromatoid bodies also occur in the cytoplasm. The mitochondria in the spermatocyte occur as small granules scattered indiscriminately in the cytoplasm and they are slightly larger than those found in the spermatogonia. The Golgi bodies are in the form of rings and crescents and lie scattered in the cytoplasm (Photograph 2). In size they are larger than those found in the spermatogonia. The primary spermatocyte stage includes a period of growth and reduction.

(*a*) *The growth period.*—This includes the preparatory stages, synapsis and tetrad-formation. The chromatin of the nucleus breaks up into small fragments and these weave out into definite leptotene threads which are distinct from each other (Fig. 13). Owing to the great number of these threads it is impossible to count them.

Synizesis now sets in. The leptotene threads get shorter and thicker and finally get shifted to one side of the nucleus (Fig. 14). Soon after this stage the cell begins to grow rapidly. The zygotene (Fig. 15) and pachytene (Fig. 16) stages have been distinctly observed and the phenomenon of parasynapsis noted. The diplotene stage (Fig. 17), wherein the threads open out at one end thereby producing figures which appear like an 8 or V or U, has been noticed. A longitudinal split soon occurs along the arms of each geminus. The two pairs of these threads diverge in opposite directions and ultimately form X-shaped structures. The tetrads are thus formed (Fig. 18). Each thin thread shortens and thickens into a spherical chromatin mass so that very soon every geminus is changed into four spherical univalent chromosomes. Next, pairs of these univalent chromosomes fuse, resulting in two

large bivalents attached to each other by linin threads. The condensation of the bivalents continues with the formation of dumb-bell-shaped structures.



With the completion of this growth period, the cell gets ready to undergo reduction.

(*b*) *The reduction division.*—The centrosome begins to divide, and the two products of division migrate to opposite poles of the cell. The nuclear wall begins to break down and the spindle is formed. The dumb-bell-shaped chromosomes are pulled to the equator of the spindle to undergo reduction (Fig. 19). Polar views of the metaphase stage (Fig. 20) show the number of chromosomes to be half that of the spermatogonial stage. The anaphase and telophase stages soon follow and the secondary spermatocytes are formed.

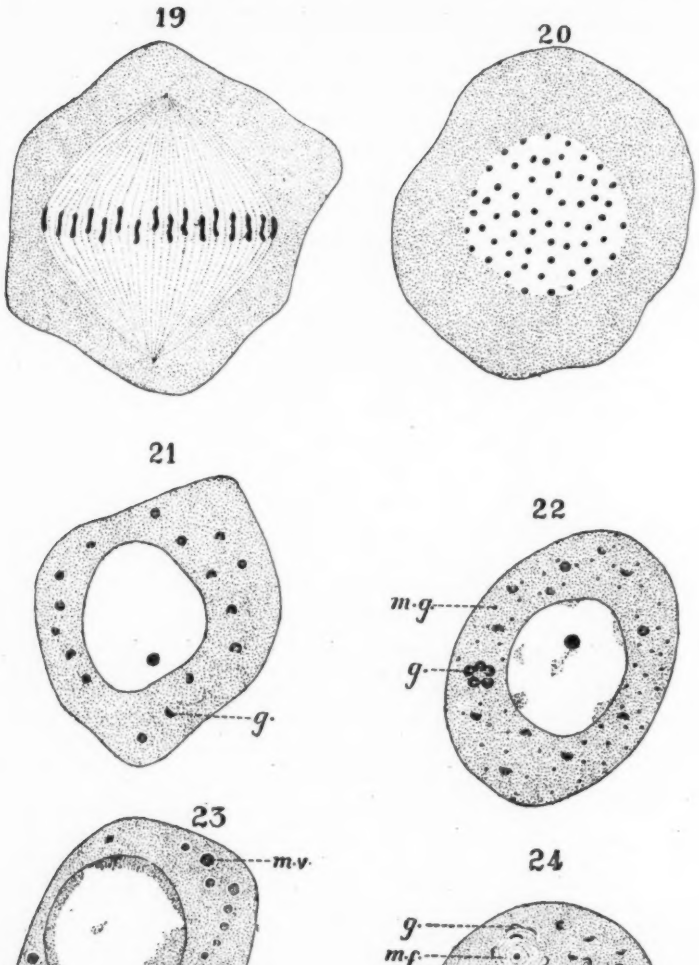
The Secondary Spermatocyte Stage

The secondary spermatocytes are about half the size of the primary spermatocytes, the chromosomes being correspondingly smaller. Numerous polar views of the metaphase stage reveal 58 chromosomes. The division of the secondary spermatocyte is equational. A chromatoid body is present in the cytoplasm. Golgi bodies are scattered in the substance of the cytoplasm (Fig. 21) and so also the mitochondria. The anaphase and telophase stages are completed with the result that the spermatids are formed.

The Spermatid and the Transformation of the Spermatid into the Mature Sperm

The spermatid is a fairly large cell with a spherical nucleus which exhibits the presence of a nucleolus situated in the meshwork of the nuclear reticulum. In the cytoplasm lie the centrosome, chromatoid body, mitochondria and golgi elements. The centrosome is a darkly-staining granule surrounded by a clear space, its position within the cell being inconstant. The mitochondria appear as granular bodies scattered in the cytoplasm. They are particularly distinct in material fixed in Champy, Flemming without acetic and Formalin and stained by Iron hæmatoxylin. In Mann-Kopsch fixed material may be observed a few golgi elements lying scattered in the cytoplasm (Fig. 22), each of which appears either in the form of a ring or crescent with a darkly staining periphery and a lightly staining interior.

Besides these structures in the spermatid cell a very peculiar body is present within its cytoplasm (Fig. 23 and M.Ph. 3). This body exhibits an almost spherical outline with a darkly chromophilic granule in the centre which sometimes occurs duplicated and surrounding which lie a series of fine concentric laminæ which present a wavy appearance. This body invariably lies close to the nuclear membrane and in fairly advanced spermatids attains immense dimensions. In many instances two or three such bodies may occur in the same cell (Fig. 24 and M.Ph. 4). In chrome-osmium fixed material the central granule or granules stain a dark hue while the surrounding laminæ



stain a lighter tinge. In Mann-Kopsch preparations the body exhibits a similar appearance except for the fact that crescentic and ring-like golgi

bodies are distinctly observed to lie in close contact with the periphery of the body forming a sort of irregular crust to the structure (Fig. 24 and M.Ph. 4). The granule within the centre is indistinct in such preparations. The body in appearance suggests a homology with the " yolk nucleus " so frequently described by various authors in the oocytes of forms like spiders, etc. This very close resemblance of the structure that I have described in the spermatid cells of *Clibanarius* to the so-called " yolk-nucleus " in the oocytes of various forms is extremely remarkable and offers for critical consideration a parallel structure in spermatogenesis, a structure which we may therefore also conveniently designate by the identical term of " yolk nucleus ". In this connection I may mention that Schmalz in 1912 described a corresponding structure in the spermatogenesis of some Ostracods.

The literature on the yolk nucleus in itself presents many variations regarding its origin, form and structure, leaving aside its fate and physiological significance in the germ cells. Balbiani (1893), Henneguy (1893), Van der Stricht (1898) and others describe it as a spherical body while Bambeke (1898) and others present the structure as an elongated body composed of finely granular material existing either in the form of a crescent or a perinuclear ring in the cytoplasm. I shall not however at present go far beyond recording my observations on the occurrence of this structure as also deal with its ultimate fate in the spermatid cell.

The yolk nucleus which makes its initial appearance in the spermatid is observed to persist through the early history of the spermatid cell and until the later stages of transformation of the spermatid into the mature sperm commence. During the early history of this spermatid cell the nucleolus is observed to extrude chromatic material into the nuclear cavity until the nucleus becomes strewn with such irregular bits of chromatin. Dissolution of the greater part of this substance is next observed to take place within the nucleus while a lesser part of it escapes as tiny granules through the nuclear membrane and exist as such within the cytoplasm (M.Ph. 5). They however soon fuse to form larger granules which present an irregular contour, and around such extruded nucleolar material mitochondrial granules align themselves to form thin wavy concentric fibrillæ until in slightly older spermatids these nucleolar extrusions with their surrounding mitochondrial fibrillæ appear very conspicuous spherical structures. The chromatin granule in the centre, as has already been said, may at times be double in which case the granules may be either equal or unequal in size. On examining spermatids of osmicated material it is observed very distinctly that golgi bodies encrust the above-mentioned duplex structure of chromatin granules and mitochondrial fibrillæ. The structure that I have described as the yolk nucleus is obviously

therefore of triple nature being composed of extruded nucleolar material in the centre surrounded by layers of mitochondrial fibrillæ and encrusted on the outside by golgi bodies. The central granule or granules as the case may be which I have described as being chromatin in nature has previously been pronounced to be such by authors such as Will (1911), Henneguy (1893). Balbiani (1883, 1893) and a few others though another set of authors maintain that these granules are of the nature of centrosomes (Chubb, 1906). A third group of authors like Dyal and Nath (1933) observe the central granules to be golgi elements. That the central granules of the so-called " yolk nucleus" in the spermatid cells of *Clibanarius* are not centrosomal in nature is obvious from the fact that in certain cells more than one " yolk nucleus " is present each with one, two or even more granules in the centre. The occurrence of more than two centrosomes in a cell has never so far been observed, and if the large number of central granules in the " yolk nuclei " of a single spermatid cell is considered it will be perfectly obvious that these granules are not centrosomal in nature. Besides this fact, the centrosome of the spermatid has been observed in the cytoplasm lying distinct from the " yolk nucleus".

Regarding the golgi nature of the granules, as expressed by other authors, my observations do not support their view in that the components of the " yolk nucleus" are very well preserved in material fixed in Champy-kull and stained by Champy-kull, Iron hæmatoxylin and Benda's stains all of which show distinctly the differential nature of the respective parts. Flemming without aeetic also preserves the " yolk nucleus " distinctly while a nuclear fixative such as Flemming with acetic preserves the central granules in a remarkable manner contrary to what we should expect if the granules were of the nature of golgi.

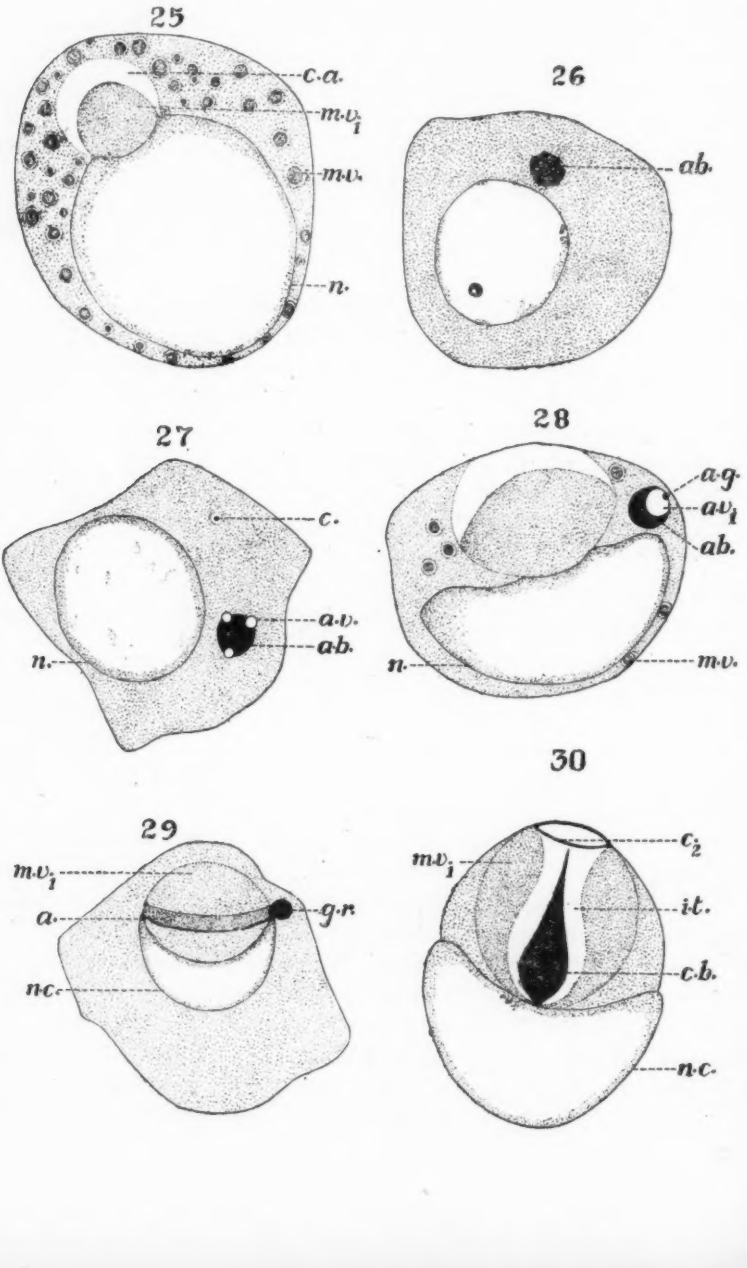
The " yolk nucleus " persists until a very late stage of the spermatid and then gradually makes its disappearance. Before its disappearance however a granular crescentic cap of mitochondria may be observed lying in a juxta-nuclear position so that invariably these two structures, the yolk-nucleus and the mitochondrial cap, may be observed in the same cell (Fig. 24 and M.Ph. 6). In Mann-Kopsch preparations discrete golgi bodies may also be observed lying in the cytoplasm. It may thus be observed that besides the golgi bodies and the mitochondria that go to constitute the yolk nucleus there are discrete golgi bodies and a mitochondrial cap present in the cytoplasm as also scattered mitochondrial granules. The mitochondrial cap by disruption later gives rise to fresh mitochondrial granules which very soon assume a scattered appearance.

Regarding its functional significance the relative size difference between a very young spermatid and a comparatively full grown transforming spermatid, and the fact that just before the spermatid ultimately resolves into the sperm the disappearance of the so-called " yolk-nucleus " is effected, suggests the explanation that the growth of the spermatid has something to do with the elaboration and ultimate disappearance of this unique structure. The spermatid in this form attains immense dimensions during its later stages of transformation into the mature sperm as compared with its very early stages and taking this fact into consideration we may, for the present, assume that the probable function of the yolk nucleus is to provide nourishment, directly or indirectly, for the transforming and developing spermatid. This assumption is purely hypothetical and shall be considered in greater detail in a later paper.

With the transformation of the spermatid the chromatoid body gradually disappears. The scattered mitochondrial granules swell into small vesicles and later fuse together to form larger ones their number consequently decreasing steadily (Fig. 25). These mitochondrial vesicles show a clear area immediately surrounding their periphery, probably a result of contraction caused by fixation of the mitochondrial vesicles. Ultimately the mitochondrial vesicles fuse together to from a single large mitochondrial vesicle which is more or less globular in form and settles on one side of the vesicular nucleus (M.Ph. 7). This large mitochondrial vesicle also at this stage exhibits a clear area around it though in the later stages it is absent due probably to the greater resistance of the mitochondrial vesicle to the action of the fixatives.

The Golgi bodies which in the early spermatid were present in the form of a few discrete, circular rings and crescents undergo progressive fusion until a single mass—the acroblast—is formed (Fig. 26). This structure evidently represents " the product of the direct reconstitution of the spermatocyte Golgi bodies". It is usually developed very near the nuclear wall (Fig. 26). In connection with the Golgi mass a number of small vesicles—the acrosomal vesicles—are soon formed (Fig. 27 and M.Ph. 8), which coalesce into a single big vesicle,—the acrosomal vesicle—in relation with which, a granule—the acrosomal granule—is recognized (Fig. 28 and M.Ph. 9). When the acrosomal vesicle is fully formed it is observed to have attained huge dimensions. It is then deposited with the enclosed granule on that part of the nuclear wall which is nearest the mitochondrial vesicle. The activity of the acrosomal granule in particular has not been followed, but the duplex product of the acroblast—the acrosomal vesicle with the enclosed granule—after it is deposited on the nuclear wall does not preserve its clear vesicular

nature and very soon the recognition of the acrosomal granule within the vesicle becomes difficult the acrosome as a whole staining rather deeply. As the transformation of the spermatid proceeds the product of the acroblast



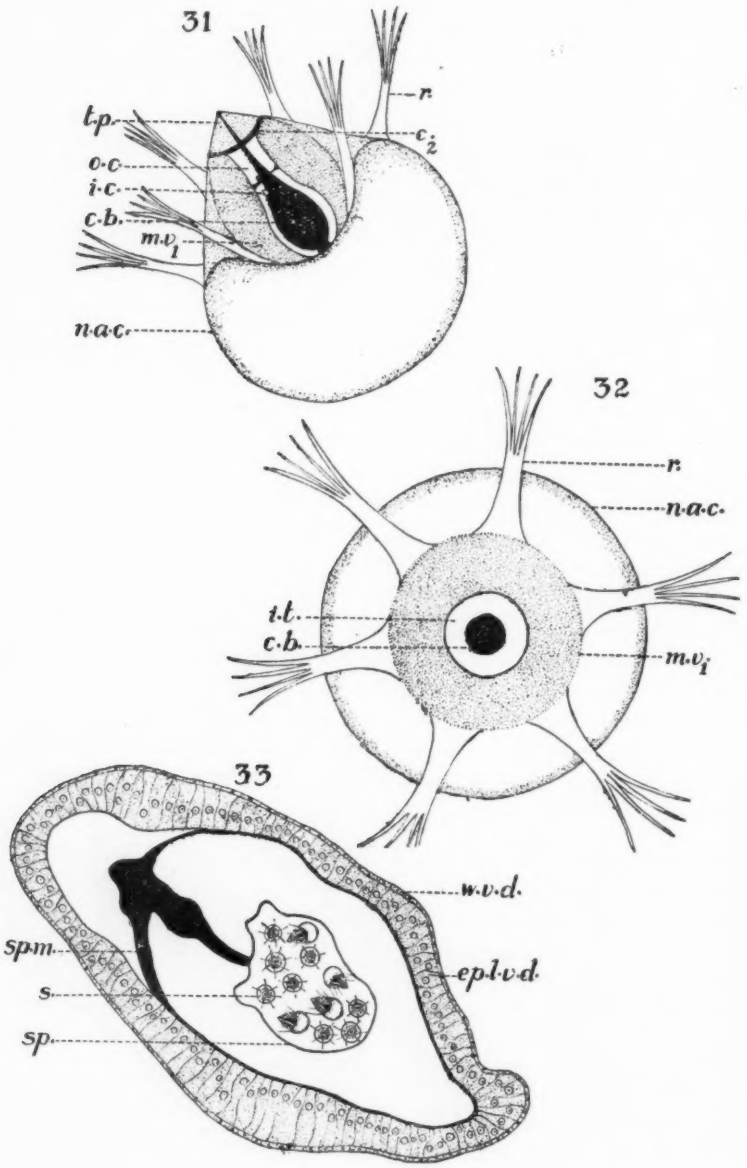
rapidly grows around the nucleus until it forms a complete ring (Fig. 29 and M.Ph. 10). With the formation of this ring which is really the acrosome of the mature sperm the nucleus becomes cup-shaped on that side of it where the mitochondrial vesicle has settled down (Fig. 29 and M.Phs. 9 and 10) and thus gradually the acrosome comes to lie on the rim of the nuclear cup and ultimately fuses with it completely so as to form a nuclear-acrosomal cup. With the completion of the ring-like acrosome, that part of the acroblast which is left in the cytoplasm after the deposition of the acrosome on the nuclear wall and which is now known as the Golgi remnant (Fig. 29 and M.Ph. 10) recedes from the nuclear wall and probably gets resorbed in the cytoplasm, no traces of the sloughing off of the Golgi remnant being detected by me in any of my peparations.

Simultaneous with the above-mentioned changes the centrosome travels towards the base of the mitochondrial vesicle and places itself in between the mitochondrial vesicle and the nuclear cup. It soon divides into two of which the lower one, now known as the proximal centrosome, grows a fairly stout, elongated and conical structure—the central body—, corresponding to the axial filament of flagellate sperms. This central body carries the distal centrosome at its extremity in its growth towards the outer border of the mitochondrial vesicle and during this process the distal centrosome at first assumes a funnel-like appearance which then breaking away its connection with the central body develops into a ring-like structure (Fig. 30) and corresponds with the " chromatin ring " of Fasten. After this period of activity on the part of the centrosomes a slight depression just within the centrosomal ring appears on the distal side of the mitochondrial vesicle. This depression sinks deep down into the mitochondrial vesicle and surrounding the central body ultimately forms a tubule (Fig. 30) which in the mature sperm, corresponds to the " secondary vesicle " of Fasten and the " inner tubule " of Binford respectively.

The central body now grows further and very soon extends beyond the ring-like distal centrosome, the cytoplasm following its course so as to give this terminal region of the developing sperm a conical appearance. This region may conveniently be designated as the " terminal piece " and may be compared to the " main piece " of the flagellate type of sperm. The inner tubule soon develops a shelf-like projection towards the central body at about the middle of its length so that in the maturing sperm the inner tubule is divided into an inner cavity and an outer cavity (Fig. 31).

The radial arms of the spermatozoon now make their appearance (Figs. 31 and 32). They vary from five to seven in number and start from the

region surrounding the nuclear-acrosomal cup radiating therefrom in a uniform manner in the shape of stout, elongated branched spines.



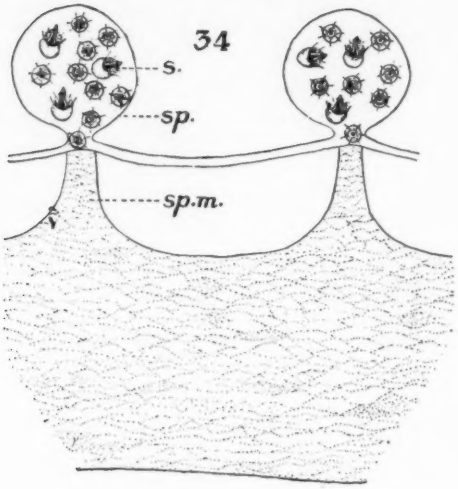
The Spermatozoon

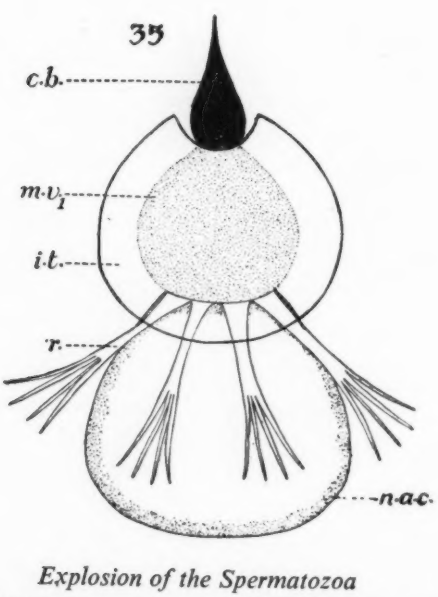
The fully formed spermatozoon is a complete disc when viewed from the bottom (Fig. 32). The margin of the disc is formed by the nuclear-acrosomal cup, though the acrosomal ring is not distinct in fresh preparations. This encloses the mitochondrial vesicle while the centre is occupied by the inner tubule within which the deeply staining central body is placed. The radial arms in the form of stout, branched spines extend outwards from the region surrounding the nuclear-acrosomal cup. In a side view of the sperm (Fig. 31) may be distinguished the nuclear-acrosomal cup, the acrosomal part however not being recognizable in fresh preparations, the mitochondrial vesicle enclosing the inner tubule, within which may be distinguished the central body extending in between the proximal and distal centrosomes and finally the " terminal piece " at the distal extremity of the sperm. The radial arms are also recognizable and in a sperm fixed in Nassanov the acrosome may be clearly distinguished extending in the form of a ring round the mitochondrial vesicle and lying fused with the rim of the nuclear cup.

The Spermatozoa in the Deferent Duct

The mature spermatozoa pass from the tubules of the testis into the deferent ducts each of which is a long convoluted tube lined with columnar epithelium (Fig. 33). The anterior end of the vas deferens is narrow and the lumen very constricted, the duct being filled with loose parenchymatous tissue. Posteriorly the lumen of the duct is wide and in this lumen are arranged the spermatophores in a single row attached to peaked processes of a membrane lining the duct (Fig. 33). The epithelium of the duct secretes a substance of the nature of mucus which hardens round a group of mature sperms in the duct and forms packets known as spermatophores (Fig. 33). The spermatophores are connected with each other by thin narrow tubes formed by extensions of the walls of the spermatophores on either side. When the duct is teased in water the membrane with the attached spermatophores comes out (Fig. 34); but the thin tube-like extensions between the spermatophores soon get ruptured due to the distension of the membrane which takes place when it comes in contact with water leaving two tag-like processes at the base of each sperm packet. When the duct is split open the membrane is seen to extend right round the row of spermatophores forming a lining to the deferent duct (Fig. 33). The ducts as well as the spermatophores are filled with mucus.

The number of sperms present in each spermatophore varies, twelve to thirty being found in each. No free sperms or free spermatophores are found in any part of the duct.





Explosion of the Spermatozoa

When mature spermatozoa are placed in dilute solutions of salts like sodium chloride, potassium chloride, calcium chloride, sodium nitrate, potassium nitrate, etc., they explode, the explosion effecting a considerable change in their appearance (Fig. 35). As Koltzoff suggested in 1906 this explosion

of the spermatozoon is probably due to osmotic pressure. The spermatozoon in its fully exploded condition has its inner tubule everted completely over the mitochondrial vesicle and the central body projecting outwards in a spine-like manner from the everted end of the inner tubule. The nuclear-acrosomal cup remains as a spherical structure at one end of the exploded sperm.

Discussion I

A. The chromatoid bodies.—The presence of a chromatoid body in the spermatogenesis of *Pentatoma* was first recognized by Wilson in 1913 and ever since similar structures have been described in the spermatogenesis of other forms. Fasten described the presence of chromatoid bodies in the seminal cells of *Cambarus virilis* and *Cancer magister* in 1914 and 1918 respectively and later in 1920, Taku Komai demonstrated the existence of chromatoid bodies in the spermatogonial cells of *Squilla oratoria.* Nath (1932) in connection with his work on the sperm cells of *Paratelphusa spinigera*, recorded the absence of chromatoid bodies, and discussed the statement made by Fasten on the existence of chromatoid bodies in the testicular cells of *Cancer.* He described the presence of a typical Golgi-idiosome complex in the spermatogonial cells of *Paratelphusa* and therefore believed that what had been described by Fasten as the two chromatoid bodies in *Cancer*, were probably not chromatoid bodies but Golgi granules which in spite of the acetic acid used by Fasten happened to escape destruction and thus presented themselves as chromatoid bodies.

In the preparations of the testicular cells of *Clibanarius* it is observed that distinct chromatoid bodies occur in the cytoplasm of the spermatogonia, spermatocytes and spermatids. In material fixed in Bouin and Carnoy they are distinct. In Champy as well as Flemming without acetic preparations of spermatogonial and spermatocyte cells, large, darkly-staining granules surrounded by clear areas are distinguished. They are distinct from the Golgi elements which exist in the very same cells as discrete, minute, circular rings or crescents in the cytoplasm. The chromatoid bodies vary in size and number and they disappear when the transformation of the spermatid commences unlike the Golgi bodies which remain and fuse together to form the acroblast in the spermatid. On a thorough examination of my preparations it occurs to me that Nath erred in suggesting that the chromatoid bodies described by Fasten in *Cancer* are not chromatoid bodies as such but Golgi granules that have escaped destruction by acetic acid. Golgi bodies and chromatoid bodies do exist in the seminal cells of *Clibanarius*, both the elements occurring side by side in the same cells, the chromatoid bodies gradually disappearing with the transformation of the spermatid, while the

Golgi bodies fuse together to form the acroblast which later secretes the acrosome of the mature sperm.

B. The nutritive cells.—The origin of the nutritive cells was a subject of discussion even so early as 1878 when Grobben first studied the sex organs of the Decapoda, and the question has continued to receive attention down to the present day. Grobben believed that the spermatogonia were derived from the nutritive cells by an increase in the size of the cell, the nucleus becoming spherical and later getting surrounded by a distinct mass of cytoplasm.

Sabatier (1885 and 1893) paid special attention to the origin of the spermatogonia while studying the germ cells of various Crustaceans such as *Astacus fluviatilis*, *Pagurus striatus* and many other forms. The spermatogonia were by him named the "Protospermatoblastes" and according to him they were derived from the cells of the replacement layer found in the testis tubules, these in turn being derived from the connective tissue layer of the tubule. The nutritive cells, he said, were then formed by a disintegration of the spermatogonial cells.

Gilson's comparative study of the spermatogenesis of the Arthropods (1886) led him to the view that the " metrocytes " or " mother cells ", were derived from the syncitial lining of the wall of the testis tubule and that the spermatogonial cells were formed by a transformation of the nutritive cells.

Keppen (1906), in his paper on the spermatogenesis of *Astacus fluviatilis*, lays particular emphasis on the relation of the spermatogonia and the nutritive cells to the germinal epithelium. He distinguishes three types of cells in the testis tubules. 1. Nuritive cells. 2. Spermatogonial cells. 3. Cells which are intermediate between these two, and he attributes a common origin to these three types of cells being derived from the germinal epithelium of the testis. He further goes on to say that these nutritive cells may often be derived from the spermatogonial cells by a process of fragmentation of the nucleus and disruption of the nuclear wall, the nutritive cells then lying within a mass of protoplasm forming a syncitium.

Herrmann, like Grobben, derived the spermatogonia from the nutritive cells after a study in 1890 of forms like *Astacus fluviatilis*, *Maja squinado*, *Eupagurus bernhardus*, *Homarus vulgaris*, *Galathea strigosa* and *Crangon vulgaris*.

Fasten, working on the spermatogenesis of the American crayfish, *Cambarus* in 1914 makes the following observations. To quote his own words: " During the period of active proliferation, the tubules in the blind ends of the testicular lobes are filled with spermatogonial cells in all stages of

development. Many nutritive cells are also found imbedded in a mass of protoplasm representing a syncituum. These last cells (nutritive cells) appear oval, irregular or circular in shape and contain masses of chromatin distributed throughout the interior. In some cases, the nutritive cells closely resemble the nuclei of the spermatogonia and it appears not at all impossible that the spermatogonia may be derived from the nutritive cells by becoming surrounded with a mass of cytoplasm as Grobben (1878) and Herrmann (1890) have claimed. In other cases spermatogonia are seen in which the cytoplasm appears to be disintegrating, thus bearing the nucleus and this might lead one to the conclusion of St. George (1892) and Keppen (1906) that some of the spermatogonia do not undergo further development, but disintegrate again into nutritive cells. The investigator cannot assert definitely as to the origin of the nutritive cells and spermatogonia. But he is inclined to the view advocated by Sabatier (1885) and Keppen (1906) that both of these cellular structures originate from the germinal epithelium of the testis and that some of the nutritive cells may then be derived from a disintegration of the spermatogonial cells."

In a later paper by Fasten (1918) wherein he describes the spermatogenesis of *Cancer magister*, he traces the origin of the nutritive cells to a definite transformation of the spermatogonia.

Taku Komai (1920) in his account of the spermatogenesis of *Squilla oratoria* states that the spermatogonia and nutritive cells have a common origin and that no nutritive cells transform into spermatogonial cells.

On reviewing the literature in regard to the origin of the nutritive cells it is observed that authors differ very widely on this point.

In the preparations of the testis of *Clibanarius*, the presence of these so-called nutritive cells alongside with the spermatogonial cells has been observed, the former being easily distinguishable from the latter by their greater capacity to take up basic dyes and also by the shape of their irregular nuclei. Evidently both these types of cells seem to be derived from the germinal epithelium lining the tubules of the testis. It has moreover been observed that the cytoplasm surrounding certain spermatogonial cells exists in a state of disintegration and dissolution, and the occurrence of such disintegration of cells leads one to believe that the nutritive cells can also be formed as a result of the disintegration of the spermatogonial cells which are not in a suitable state for further growth and advance in development. The nutritive cells are very often met with in a cluster lying in a syncitial mass of protoplasm, formed, it may be believed, by the disintegration and dissolution of the cytoplasm surrounding groups of spermatogonial cells.

C. The acrosome.—The acrosome in *Paratelphusa spinigera* is described by Nath (1932) as a ring-shaped structure formed as a result of the direct transformation of the Golgi elements present in the spermatid and lying fused with the rim of the nuclear cup. The spermatid is described as a spherical structure with a granular nucleus, the Golgi elements existing in the cytoplasm in the form of minute rings each with a darkly-staining periphery and a lightly-staining interior. With the transformation of the spermatid the Golgi elements fuse together to form a compact body which is destined to form the ring-like acrosome of the mature sperm. Nath, in his description, does not make any mention of either an acrosomal vesicle or a Golgi remnant on the belief that an acroblast which is usually said to secrete the acrosome of the mature sperm, does not exist as such in the spermatid, but, that the Golgi elements after a preliminary fusion between themselves directly get transformed into the ring-shaped acrosome, no part of the Golgi material being left behind in the spermatid as the Golgi remnant. Nath is of opinion that the acrosome in *Paratelphusa* is not formed as a secretion product of an acroblast which is formed by the fusion of the Golgi bodies, but that it is the result of a direct transformation of the Golgi bodies present in the spermatid.

Muthuswamy Iyer (1933), working on the spermatogenesis of *Paratelphusa hydrodromus*, draws a similar conclusion in regard to the formation of the acrosome.

On a thorough examination of the spermatid cells of *Clibanarius*, structures of the nature of acroblasts have been observed formed as a result of the fusion of the Golgi bodies in the spermatid cells. These are prominently present in material fixed in Champy and Flemming without acetic and stained with Iron hæmatoxylin. They are also frequently observed in sections made by the method of Ludford. The acroblast soon assumes the form of a deeply staining crescent in association with which a non-staining vesicle, —the acrosomal vesicle—enclosing the acrosomal granule is found to be developed. In a few cases a number of vesicles are seen to be associated with a deeply staining part, the whole forming a single mass. Later all the vesicles join together to form a single big vesicle within which is the acrosomal granule. This duplex product of the acroblast—the acrosomal vesicle with the enclosed granule—is eventually deposited on the nuclear wall and develops into the ring-shaped acrosome. When the acrosome is in the process of development into the ring-like form that part of the acroblast which is left in the cytoplasm after elaboration of the acrosome and which is now known as the Golgi remnant thence forward gradually recedes from the nuclear wall, and it seems probable that this Golgi remnant gets finally

resorbed in the cytoplasm of the transforming spermatid. The complete casting off of the Golgi remnant from the spermatid has not been observed and as such it may be believed that the acroblast after secreting the vesicular acrosome and depositing it on the nuclear wall recedes from its position and finally gets resorbed in the cytoplasm.

In this connection it may be said that it is very probable that Nath erred when he expressed the view that the acrosome in *Paratelphusa spinigera* is formed by the direct transformation of the Golgi elements present in the spermatid. No previous author has, to my knowledge, finally described the formation of the acrosome as being the result of the direct transformation of the Golgi elements and Bowen in 1922 draws the important conclusion from his observations " on the idiosome, Golgi apparatus and acrosome in the male germ cells " that " the acrosome is a secondary product of the acroblast and that neither idiosomic nor Golgi material goes directly into its formation ". The view of all recent workers on spermatogenesis seems to be at present that the acrosome is a secretory product of the Golgi, and it is therefore highly improbable that in these two forms—*Paratelphusa spinigera* and *Paratelphusa hydrodromus*—only, the acrosome should arise in a different manner. Very much more proof will hence have to be forthcoming before this view can be finally accepted.

Discussion II

(*a*) *The mitochondria.*—While this piece of research was in progress no author had published a thorough and conclusive study of the mitochondria in the germ cells of the Decapoda and established its continuity from the earliest spermatogonia to the mature sperm. Koltzoff (1906), Binford (1913), Reinhard (1913) and Fasten (1918) studied the mitochondria only from the stage of spermateleosis while Grabowska's (1928) researches led him no further than the spermatogonial and spermatocyte stages. Nath (1932) made only a preliminary study of the spermatogonia and spermatocytes while making a thorough study of the spermatid cells of *Paratelphusa spinigera.*

My researches on *Clibanarius* has enabled me to trace the complete history of this cytoplasmic element from the earliest spermatogonial stage to the stage wherein the sperm is completed, and for this purpose I have employed all the recent technical methods prescribed for the correct identification of the mitochondrial element. As such I have seen and described the initial presence of the mitochondria in the spermatogonia as a crescentic cap of granular material closely apposed to the nucleus, and the Golgi bodies as a clump of dark ring-like or crescent-shaped discrete bodies in the cytoplasm. Within the spermatocytes I have traced a disintegration of this nuclear

cap of mitochondria entailing a scattered arrangement of the mitochondrial granules and later a progressive increase in size of these individual granules. It is only in the transforming stage of the spermatid that the vesicular appearance of the mitochondria is obtained, and the further formation of the large mitochondrial vesicles observed. This mitochondrial vesicle which forms the major part of the sperm of *Clibanarius* corresponds with the " capsule " of the sperms of the three species of decapods described by Worley (1939). The "Capsule" however, is described by Worley as being formed by the coalescence of cytoplasmic vacuoles present within the spermatid, while I have described the corresponding structure as being mitochondrial in origin. The mitochondrial vesicle in the sperm of *Clibanarius* and the " capsule " in the three decapod sperms studied by Worley therefore differ entirely in structure though they all seem to singularly occupy the identical region in the sperms of all the four species concerned.

Nath (1932) in working out the spermateleosis of *Paratelphusa spinigera* describes the occurrence of mitochondrial vesicles in the early spermatid cells, and these, he says, fuse later to form the big mitochondrial vesicle which lies in close proximity with the cup-like nucleus in the mature sperm. In regard to these vesicles Nath says: " In many cases the cytoplasm is so densely packed with them that it presents the appearance of a honey comb, his figures of the spermatid cells showing the mitochondrial vesicles presenting a striking similarity with the vacuolation of the cytoplasm described by Worley and depicted in her figures of the same."

The absence of a cytoplasmic " capsule " in *Paratelphusa* such as is described by Worley in the three species that she has studied is remarkable, and the observations of Nath (1932) tallying with what occurs in *Clibanarius* that I have studied, it may be said that Worley very probably misinterpreted the " capsule" as being cytoplasmic in origin. My observations on *Clibanarius* have shown the existence of a definite deeply staining cresentic mitochondrial mass in the spermatogonium which being followed through in the spermatocyte and spermatid stages have shown the disintegration of the cap with the formation of scattered granules in the former which in the latter swell into vesicles and eventually fuse to form the spherical structure to be ultimately designated as the mitochondrial vesicle of the mature sperm. Towards the formation of this vesicle all the small mitochondrial vesicles progressively contribute until in the mature sperm no small mitochondrial vesicles are visible unlike that described by Worley in the hermit crab, *Pagurus*, where some of them remain as discrete bodies located between the capsule and the nucleus. I have furthermore never observed any process of cytoplasmic vacuolation in material which preserved mitochondrial

vesicles though in preparations where with the employment of acetic acid the mitochondrial elements were dissolved out clear empty spaces were visible giving the cytoplasm a vacuolated appearance. Besides, no previous author has as yet described the parallel occurrence of such a structure as the cytoplasmic " capsule " in the flagellate sperms and it seems very strange and unaccountable why such a structure as the cytoplasmic " capsule " should ever be present in the non-flagellate sperm at all, and though Worley ascribes the shape of the sperm to the presence of this unique structure it seems incomprehensible that such a structure as the cytoplasmic " capsule " should be produced within the sperm only to give the sperm its characteristic shape.

Considering the flagellate sperm it will be observed that the elongate nature of the sperm is entirely due to the mitochondrial envelope and the axial filament both of which ultimately assume a very attenuated form, and therefore it seems questionable why the axial filament and the mitochondrial sheath of the flagellate sperm which together are responsible for its elongate nature should not equally be responsible for the shape of the radiate type of sperm such as occurs in *Clibanarius* and the three forms studied by Worley. Setting aside this probability it may be recalled that one of the recent contributions to science has been the generalized fact that " the mitochondria (or their product) always form an envelope atleast of some part of the axial filament " (*vide* Nath, 1932), and since this fact identically reveals the condition present in *Clibanarius* I strongly feel that the " capsule " of the decapod sperms studied by Worley is a misinterpreted structure and that its real homology lies in the mitochondrial sheath of the flagellate sperm.

Koltzoff (1906) has suggested that the " capsule " of the decapod sperm serves a mechanical function. It may be that it is so and besides being obviously responsible for the particular shape of the sperm it may be suggested that this mitochondrial part of the non-flagellate sperm may also subserve a nutrient function.

The mitochondria according to Worely give rise to either a mitochondrial ring as in *Pagurus* and *Libinia* or to a triangle as in *Homarus* from which radial processes develop and support the arms of the respective sperms. I have however, contrary to Worley's observations, observed the arms in *Clibanarius* to be entirely of cytoplasmic origin, the mitochondria constituting only the mitochondrial vesicle and not contributing to the formation of the radial arms at all.

(*b*) *The centrosome.*—The structure and behaviour of the centrosome has been very clearly followed through in all the stages of spermatogenesis

of the flagellate sperm by many authors, and the fact that the axial filament takes its origin from the centrosome is well established. Such being the case we would naturally expect a parallel to this occurrence in the non-flagellate sperm as well.

From what has been described of the three species of decapods by Worley it is evident that in *Homarus* and *Pagurus* a simple rod-shaped structure, the axial rod, develops from the proximal centriole and in *Libinia* the proximal centriole is modified into a long medial process which however, takes the opposite direction of that of the axial rod. That the axial filament in flagellate sperms originates from the centrosome has been determined by many authors though opinions differ as to whether it is the proximal or distal centrosome that gives rise to the axial filament. In the form *Clibanarius* that I have studied, I have observed the central body (axial rod) to develop from the proximal centrosome and the distal centrosome to give rise to the centrosomal ring at the extremity of the mitochondrial vesicle (*vide* Nath, 1932). In the behaviour of the proximal centrosome the three species described by Worley seem to closely resemble each other though all the three species exhibit at the same time differential behaviour of the distal centrosome. However, in *Pagurus* Worley describes the distal aperture as being distinctively formed by the distal centriole in which case it closely approximates the distal centrosomal ring in *Clibanarius*.

In a typical flagellate sperm it is quite often observed that the proximal centrosome gives rise to the axial filament, while the distal centrosome assuming a ring-like structure slips down the axial filament and occurs as such at the posterior end of the mitochondrial sheath. In *Clibanarius* the behaviour of the proximal and distal centrosomes is very closely similar to what occurs in a flagellate sperm and from the fact that *Pagurus* helps to substantiate what occurs in *Clibanarius* (assuming that the "capsule" of *Pagurus* is homologous with the mitochondrial vesicle of *Clibanarius*) I am inclined to adopt the view that the activity of the centrosome in both flagellate and non-flagellate sperms is similar. The mitochondrial sheath of the flagellate sperm, as is well known, is limited at its two extreme ends by the proximal and distal centrosomes and this condition is paralleled closely in such a non-flagellate sperm as that of *Clibanarius* which has at either extremities of its mitochondrial capsule the two centrosomes, proximal and distal, identically similar to what obtains in flagellate sperms in general. Another point of resemblance in this connection is the inclusion of the axial filament within the mitochondrial sheath in the flagellate sperm and the inclusion of the central body (axial rod) within the mitochondrial capsule in the non-flagellate sperm, the two structures, the axial filament as well as the

central body being derived exclusively from the proximal centrosome in both cases. In *Homarus* also the proximal centrosome is deputed for the formation of the axial rod though in *Libinia* a slight deviation is observed in that part of the distal centrosome gives rise to the axial rod while the proximal centrosome gives rise to an extraordinary structure—the medial process—to which no function could be attached with certainty.

From my observations on *Clibanarius* and those of Worley on the three species of Decapods mentioned it may be possible, with the existing knowledge of flagellate sperms, to draw certain generalizations regarding the activity of the centrosome which may equally well apply to both flagellate and non-flagellate sperms in the animal kingdom. Considering the two types, it is observed that the mitochondrial region of the sperm is the main feature that gives to the sperm its characteristic shape, though the axial filament or the axial rod that it encloses may be the factor that predetermines the shape of the mature sperm. Whatever that may be, we ultimately observe that it is the mitochondria and the two centrosomes, the proximal and the distal, that restrict the boundaries of the mitochondrial sheath or vesicle as the case may be and give to the sperm its ultimate form. Taking this fact into consideration we may with greater strength support the statement that I have made in the previous section regarding the cytoplasmic vesicle in the sperms of the three species of decapods studied by Worley, that it is of mitochondrial origin and not a mere cytoplasmic structure as depicted by this author. If the structure that has been designated as the " capsule " by Worley and pronounced to be of cytoplasmic origin is of mitochondrial nature, we may safely ascribe a particular function to the mitochondrial structure which takes the form of an attenuated sheath in the flagellate sperm and that of an almost spherical vesicle in the non-flagellate sperm, as well as to the two, division products of the centrosome, the proximal and the distal, in that all these elements participate in moulding the exact and ultimate form of the mature sperm.

(*c*) *The acrosome.*—Worley's study of the three species of decapods has led her to the interpretation that the central tube in all the three species and the distal aperture of the central canal in *Homarus* and *Libinia* are acrosomal in nature. My observations on the decapod, *Clibanarius*, have however led me to a different interpretation of the corresponding part and in this section I make an attempt to show what to me has seemed the most probable solution of the problem in discussion.

Despite the fact that Bowen (1925) has expressed the idea that no homology in regard to the topographical structure of the flagellate sperm with that

of the non-flagellate sperm could be traced, and that the only way to look at the non-flagellate sperm is to find in it the corresponding equivalents of the component parts of the flagellate sperm, however the respective parts may be arranged in the two types of sperms, no previous work on spermatogenesis has as yet revealed the deposition of the acrosome in relation with any structure other than the nucleus. To quote a few passages from Bowen (1924): 1. "The acrosomal material is deposited by the acroblast (golgi complex) at some point on the nuclear membrane, usually before the nucleus has changed (in case the sperm head is to be elongate) from its original spherical shape"; 2. "The shape of the sperm head and the relation of the acrosome to it are the important points"; 3. "The bulk of the acrosome may be located almost anywhere on the nuclear periphery"; and again in 1925: 4. "The golgi complex gives rise to the acrosome which is always associated in one way or another with the nucleus in the head region of the sperm". From this point of view, therefore, Worley's conclusions regarding the acrosome in the three decapod species need some consideration.

It will be observed that Worley has recorded the formation of the acrosome in the three species of decapods studied always in connection with a cytoplasmic structure known as the "capsule"—a structure the equivalent of which has never so far found a place in any flagellate sperm. Though Bowen (1925) distinguishes the fact that non-flagellate sperms are not foreshortened flagellate sperms as Koltzoff (1906) and others have explained and that the non-flagellate sperm "merely represents a way of putting certain essential components into a compact and convenient packet" (*vide* Bowen, 1925), in all cases of non-flagellate sperms where the process of acrosomal differentiation has been studied no instance can be cited where it is developed in connection with any structure other than the nucleus. In flagellate sperms it is the same. In whatever position the acrosome may be placed in the ultimate sperm it always shows a close relationship with the nucleus. When we take even an atypical flagellate sperm like that of *Lepisma* into consideration, this relationship between the nucleus and the acrosome is still seen to be preserved even though the sperm as a whole may present a very unique form and structure. It is only in the forms mentioned by Worley that this relationship between the nucleus and the acrosome is not maintained, the acrosome in all the three cases being deposited from the initial stages of its differentiation on the periphery of such an unusual structure as the "capsule" and retaining this relationship throughout the period of spermateleosis, though, in the final product of spermatogenesis, the acrosome develops a form peculiar to itself and strikingly different from what it was at the beginning.

In my observations on the spermatid cells of *Clibanarius* I have described the acrosome as a ring-like structure fused with the margin of the nuclear cup, formed in the initial stages as a vesicular secretion of the acroblast which being deposited on the nuclear periphery grows round and ultimately attains its ring-like form. The position occupied by the cytoplasmic " capsule" in the sperms studied by Worley is here filled by the mitochondrial vesicle while the mitochondrial processes described by Worley are accounted by me as cytoplasmic in origin. How could this difference in interpretation be satisfactorily explained ?

When we take the structure of the sperm of *Clibanarius* and that of a flagellate sperm into consideration we are as it were struck by the almost identical similarity between the parts of the two types of sperms. The nucleus forms the head to which the acrosome is attached though the mode and place of deposition of the acrosome may be different in the two forms, the proximal centrosome follows the head from which the axial filament takes its origin in the flagellate sperm while the corresponding central body or axial rod develops in the non-flagellate sperm. This axial filament or the central body as the case may be is surrounded by the mitochondrial sheath in the flagellate sperm and the corresponding mitochondrial vesicle in the non-flagellate sperm; the distal centrosome in the form of a ring occupies the farthest end of the mitochondrial sheath in the flagellate sperm while in the non-flagellate sperm of *Clibanarius* the same structure, taking on an identical form, occupies the corresponding distal end of the mitochondrial vesicle. The main piece together with the end piece of the flagellate sperm finds its counterpart in the " terminal piece " of the non-flagellate sperm of *Clibanarius*. We thus see that there is a remarkable resemblance between the two types of sperms, the flagellate and the non-flagellate (*vide* Nath, 1932) and despite Bowen's statement that such a topographical similarity between flagellate and non-flagellate sperms is of no significance whatsoever, even if it does exist at all, and that real similarity between sperms should be traced only in their component parts, the fact that no sperm has as yet been figured where the acrosome is shifted so far away from the nucleus would draw us nearer to the conclusion that Worley probably misinterpreted the actual formation of the acrosome in the forms that she had studied.

In conclusion, I may say that I have not observed the radial arms of the spermatozoon in *Clibanarius* to be of mitochondrial origin contrary to what has been described by Worley for the three decapod species studied by her. In studying the mitochondria I have traced their occurrence from their initial stages in the spermatogonia where they exist as a compact and crescentic cap of granular substance, through the spermatocytes wherein the

mitochondrial cap disrupts with the formation of scattered granules in the cytoplasm which after further growth and development into small mitochondrial vesicles fuse to form the big mitochondrial vesicle in the spermatid. The activities of the mitochondria being so thoroughly followed through in all the stages of spermiogenesis I cannot for a moment believe that I could possibly have overlooked the exact formation of the mitochondrial vesicle which finds its counterpart in regard to its position in the cytoplasmic " capsule " of the sperms figured by Worley. The position and relationship of the centrosomes to the mitochondrial vesicle add support to my statement that what I have depicted as the mitochondrial vesicle is really of mitochondrial nature and finds its homology in the mitochondrial sheath of the flagellate sperm. The secretion and mode of development of the acrosome have also been systematically studied, and the fact that it is found associated with the nucleus from its earliest stage of deposition like that described for all sperms except those described by Worley, represents to my mind that my account gives a correct picture of the real state of things. I may here relevantly recall the observations of Nath (1932) on the spermatid cells of *Paratelphusa spinigera* where the development of the acrosome is traced in an almost similar manner except for the fact that he describes direct transformation of the acroblast into the acrosome contrary to my observations on the spermatid cells of *Clibanarius*, where it is definitely formed as a vesicular product of secretion of the acroblast which ultimately gets transformed into the ring-like form in the mature sperm.

Summary

1. The testis of *Clibanarius olivaceous* is a paired elongated organ, which consists of a number of testicular tubules each of which shows the seminal elements in all the stages of growth from one end of the tubule to the other.

2. The spermatogonia and the nutritive cells originate from the germinal epithelium lining the wall of the testicular tubules. The nutritive cells also arise by the degeneration of certain spermatogonial cells.

3. Primary and secondary spermatogonia may be distinguished. The spermatogonial number of chromosomes is 116. In the cytoplasm of the spermatogonium occur chromatoid bodies, a centrosome, a crescentic granular mass of mitochondria closely applied to the nuclear wall, and also a few Golgi elements.

4. After the spermatogonial divisions the primary spermatocyte is formed which includes a period of growth and reduction. The first

spermatocyte division is reductional. The number of spermatocytic chromosomes is 58.

5. The division of the primary spermatocyte results in the secondary spermatocytes. The secondary spermatocyte division is equational, a polar view of the metaphase stage revealing 58 chromosomes.

6. Golgi and mitochondria occur as scattered elements in the cytoplasm of the primary and secondary spermatocytes.

7. By the division of the secondary spermatocyte the spermatid is formed which has an almost spherical, granular nucleus. In the cytoplasm a centrosome, chromatoid body, mitochondrial granules, a few golgi elements and a " yolk nucleus " are visible. A mitochondrial cap is also present which soon ruptures to give rise to mitochondrial granules.

8. In a later spermatid the nucleus swells and presents a vesicular appearance. Meanwhile the mitochondrial granules swell into small vesicles which fuse together to form larger ones, these ultimately fusing to form a large mitochondrial vesicle which settles on one side of the vesicular nucleus. The golgi bodies fuse together and form the acroblast which secretes a vesicular product—the acrosomal vesicle—enclosing the acrosomal granule. The yolk nucleus gradually disappears.

9. This secreted duplex product of the acroblast is deposited on the nuclear wall after which it rapidly grows and expands into a ring-like structure surrounding the vesicular nucleus. The golgi remnant probably gets resorbed in the cytoplasm.

10. The nucleus becomes slightly cup-shaped and the mitochondrial vesicle settles in the cavity of the nuclear cup. With this sinking in of the mitochondrial vesicle into the nucleus the ring-like acrosome gets fused with the rim of the nuclear cup.

11. Meanwhile the centrosome travels to the base of the mitochondrial vesicle and there divides into two of which one remains as the proximal centrosome and gives rise to the central body, which in its growth outwards carries the other centrosome to the distal end of the mitochondrial vesicle. Here it grows into a ring-like structure and comes to be known as the distal centrosome.

12. A depression is soon formed within the centrosomal ring which sinks into the mitochondrial vesicle and gives rise to a tubular structure hitherto known as the " inner tubule".

13. The central body now grows further and extends beyond the distal centrosomal ring. At the extreme tip of the sperm is present a conical

structure which fits into the cavity of the centrosomal ring. The arms of the spermatazoon grow out in the form of slender, radiating branched spines from the region surrounding the nuclear-acrosomal cup.

14. The mature spermatozoon is a spherical structure with a cup-shaped nucleus, to which is attached the ring-like acrosome along its rim, an almost globular mitochondrial vesicle, a spherical proximal centrosome and a distal ring-like one between which, and extending slightly beyond the latter, the central body exists. A conical structure occupies the extreme distal end of the sperm. Radial arms extend outwards from the region surrounding the nuclear acrosomal cup in the form of slender radiating branched spines. When the spermatozoa are ripe they are passed down the deferent ducts where they get enveloped in packets known as spermatophores.

15. When mature spermatozoa are placed in dilute salt solutions they undergo an explosion causing the mitochondrial vesicle, inner tubule and central body to be completely everted while the nuclear-acrosomal cup rounds out into a spherical structure.

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N.B.—* Have not been referred to in the original.

KEY TO LETTERING

a.	.. Acrosome.	*m. v.*	.. Small mitochondrial vesicles.
ab.	.. Acroblast.	*m.v.*[1]	.. Large mitochondrial vesicle.
a.g.	.. Acrosomal granule.	*n.*	.. Nucleus.
a.v.	.. Small acrosomal vesicles.	*nu.*	.. Nucleolus.
a.v.[1]	.. Large acrosomal vesicle.	*n.c.*	.. Nuclear cup.
c.	.. Centrosome.	*n.a.c.*	.. Nuclear-acrosomal cup.
c_1	.. Proximal centrosome.	*nu.e.*	.. Nucleolar extrusions.
c_2	.. Distal centrosome.	*o.c.*	.. Outer cavity.
c.a.	.. Clear area surrounding mitochondrial vesicle.	*p.sg.*	.. Primary spermatogonia.
		p.sg.m.p.	.. Primary spermatogonial metaphase plate.
c.b.	.. Central body.		
ch.	.. Chromatin.	*p.sg.m.s.*	.. Primary spermatogonial metaphase spindle.
ch.b.	.. Chromatoid body.		
ch.g.	.. Chromatin granule.	*r.*	.. Arms.
ep.l.v.d.	.. Epithelial lining of vas deferens.	*s.*	.. Spermatozoon.
g.e.n.	.. Germinal epithelial nuclei.	*sp.*	.. Spermatophore.
g.	.. Golgi.	*sp.m.*	.. Membrane of spermatophore.
g.r.	.. Golgi remnant.	*t.*	.. Testis.
i.c.	.. Inner cavity.	*t.f.*	.. Testicular follicle.
i.t.	.. Inner tubule.	*t.p.*	.. Terminal piece.
m.c.	.. Mitochondrial cap.	*t.t.*	.. Testicular tubule.
m.f.	.. Mitochondrial fibrillæ.	*v.d.*	.. Vas deferens.
m.g.	.. Mitochondrial Granule.	*y.n.*	.. Yolk nucleus.
		w.v.d.	.. Wall of vas deferens.

EXPLANATION OF FIGURES

N.B.—All figures except Figs. 1, 31, 32, 34 and 35 have been drawn with the camera lucida.

FIG. 1. The male reproductive system. Diagrammatic.

FIG. 2. Cross-section of a testicular follicle showing the tubules. Carnoy and iron hæmatoxylin. × 400.

FIG. 3. Resting primary spermatogonium showing the mitochondrial cap, centrosome and chromatoid bodies. Champy and iron hæmatoxylin. × 3000.

FIG. 4. Resting primary spermatogonium showing the mitochondrial cap and Golgi bodies. Mann-Kopsch and Altmann Stain. × 2000.

FIG. 5. Nutritive cell showing the nucleus with pseudopodial processes. Champy and iron hæmatoxylin. × 2000.

FIG. 6. Primary spermatogonium. Side view of metaphase showing mitochondrial granules scattered in the cytoplasm. Flemming without acetic and iron hæmatoxylin. × 2000.

FIG. 7. Primary spermatogonium. Polar view of metaphase. Carnoy and iron hæmatoxylin. × 2000.

FIG. 8. Primary spermatogonium. Side view of anaphase. Distribution of mitochondrial granules. Flemming without acetic and iron hæmatoxylin. × 2000.

FIGS. 9 & 10. Primary spermatogonia. Side views of anaphase. Carnoy and iron hæmatoxylin × 2000.

FIG. 11. Primary spermatogonium. Side view of telophase. Carnoy and iron hæmatoxylin. × 2000.

FIG. 12. Resting primary spermatocyte showing the centrosome, chromatoid body and mitochondrial granules. Champy and iron hæmatoxylin. × 3000.

FIG. 13. Primary spermatocyte. Leptotene stage. Carnoy and iron hæmatoxylin. × 2000.

FIG. 14. Primary spermatocyte. Synizesis. Carnoy and iron hæmatoxylin. × 2000.

FIG. 15. Primary spermatocyte. Zygotene stage. Carnoy and iron hæmatoxylin. × 2000.

FIG. 16. Primary spermatocyte. Pachytene stage. Carnoy and iron hæmatoxylin. × 2000.

FIG. 17. Primary spermatocyte. Diplotene stage. Carnoy and iron hæmatoxylin. × 3000.

FIG. 18. Primary spermatocyte. Tetrad formation. Carnoy and iron hæmatoxylin. × 2000.

FIG 19. Primary spermatocyte. Side view of metaphase. Flemming with acetic and iron hæmatoxylin. × 2000.

FIG. 20. Primary spermatocyte. Polar view of metaphase. Flemming with acetic and iron hæmatoxylin. × 2000.

FIG. 21. Resting secondary spermatocyte. Golgi bodies scattered in cytoplasm. Ludford. × 2000.

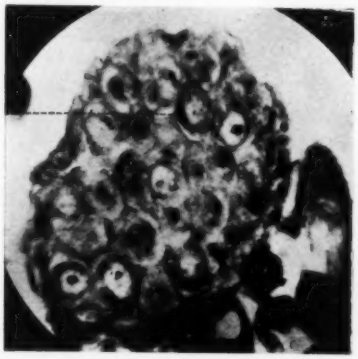
FIG. 22. Spermatid. Golgi bodies and mitochondrial granules in cytoplasm. Mann-Kopsch and Altmann stain. × 2000.

FIG. 23. Spermatid showing the presence of a yolk nucleus in the cytoplasm. Champy and iron hæmatoxylin. × 2000.

FIG. 24. Spermatid showing mitochondrial cap and two yolk nuclei, the latter showing golgi bodies at their periphery. Mann-Kopsch. × 2000.

FIG. 25. Spermatid. Formation of mitochondrial vesicles. Champy and iron hæmatoxylin. × 2000.

1



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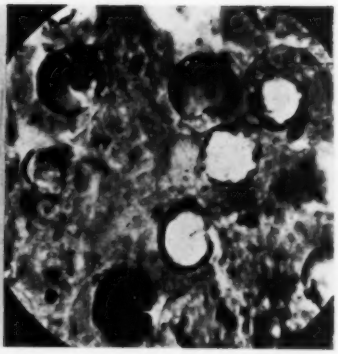

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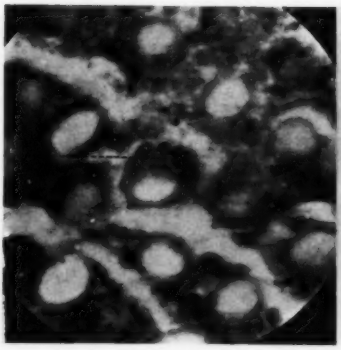

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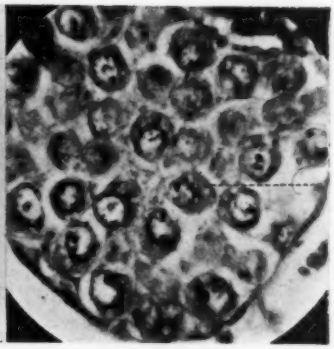

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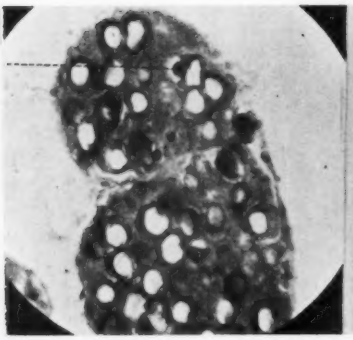

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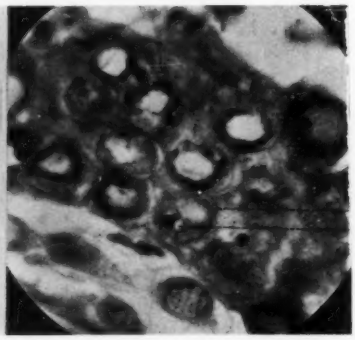

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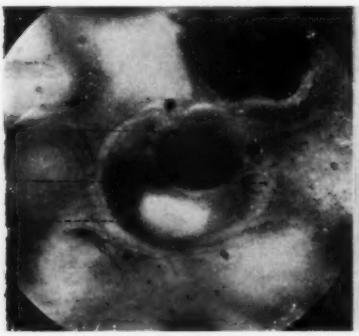

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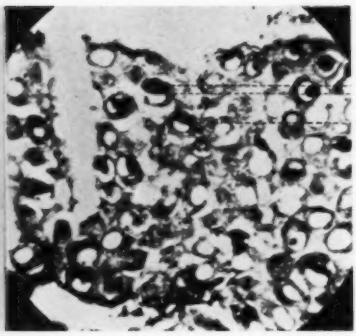

FIG. 26. Spermatid. Acroblast present. Mann-Kopsch. × 2000.

FIG. 27. Spermatid. Formation of acrosomal vesicles. Champy and iron hæmatoxylin. × 2000.

FIG. 28. Spermatid. Formation of acrosomal vesicle with acrosomal granule. Champy and iron hæmatoxylin. × 2000.

FIG. 29. Spermatid. Formation of ring-shaped acrosome. Mitochondrial vesicle, nuclear cup and Golgi remnant present. Nassanov. × 2000.

FIG. 30. Spermatid. Formation of distal centrosomal ring, inner tubule and central body. Carnoy and iron hæmatoxylin. × 2000.

FIG. 31. Mature spermatozoon. Side view. Diagrammatic.

FIG. 32. Mature spermatozoon. Bottom view. Diagrammatic.

FIG. 33. Cross-section of vas deferens. Bouin and iron hæmatoxylin. × 80.

FIG. 34. Membrane of spermatophore with spermatophores intact. Diagrammatic.

FIG. 35. Exploded sperm. Diagrammatic.

EXPLANATION OF MICROPHOTOGRAPHS

PLATE XVIII

MICROPHOTOGRAPH 1. Section of testis showing spermatogonia with mitochondrial caps. 2. Section of testis showing golgi bodies in primary spermatocytes. 3. Section of testis showing spermatids with yolk nuclei. 4. Section of testis showing two yolk nuclei in one spermatid; the encrusting golgi bodies are visible. 5. Spermatids showing extrusion of nucleolar chromatin material into the cytoplasm.

PLATE XIX

MICROPHOTOGRAPH 6. Section of testis showing spermatid with both mitochondrial cap and yolk nuclei in cytoplasm. 7. Section of testis showing the formation of the big mitochondrial vesicle. 8. Section of testis showing the formation of acrosomal vesicles. 9. Section of testis showing the formation of the acrosomal vesicle with the enclosed acrosomal granule. 10. Section of testis showing the acrosomal ring and golgi remnant in spermatids.

N.B.—Drawings reduced to three-fourth the magnifications given.

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ERRATA

Vol. XIII, No. 5, May 1941

Page 307, 1st para, 9th line: *delete* " But " and *read* " he " as " He ".

„ 307, 3rd para, 9th line: *read* " chlorella " *as* " Chlorella ".

„ 308, under C, 4th line: *delete* " It was " and *insert* " They were ".

5th line: *delete* " It " and *insert* " The whole mass ".

„ 312, 1st para, 1st line: *after* " magnitude of the rise and fall " *insert* " of total nitrogen ".

„ 314, lines 3–10 commencing with " and its synthesis . . . ." and ending with " travel to the stem " *to be read as* " and their synthesis on the other, will proceed at a rate depending upon their removal. In a young fast-developing plant, the amino acids from the leaves move to the meristematic regions in the stem. Simultaneously with their removal, on the basis of mass action, increasing quantities of this acid will be produced. Conversely, in a mature plant such as plants 3–4 months old where vegetative growth has not only ceased but the spikes have also formed, the amino acids do not travel to the stem ".

„ 315, 1st para, 1st line: *after* " 4 p.m. " *add* " on the following day ?"

„ 318, 18th line: the word " Acer " to be in italics.

„ 320, 20th line: *read* " as it affects " *as* " as they affect ".

„ 320, 24th line: *insert* " process " between " this " *and* " requiring ".

„ 320, 35th line: *read* " in the leaves " *as* " in the uninjected leaves ".

„ 321, 15th line: *read* " in excess to " *as* " in excess of ".

„ 324, Under Literature Cited, Ref. No. 2: *read* " 19 and 3 " *as* " 1923 ".

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